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Report Number 22

RESEARCH IN RADIOLOGY

Henry S. Kaplan
Editor

National Academy of Sciences—
National Research Council

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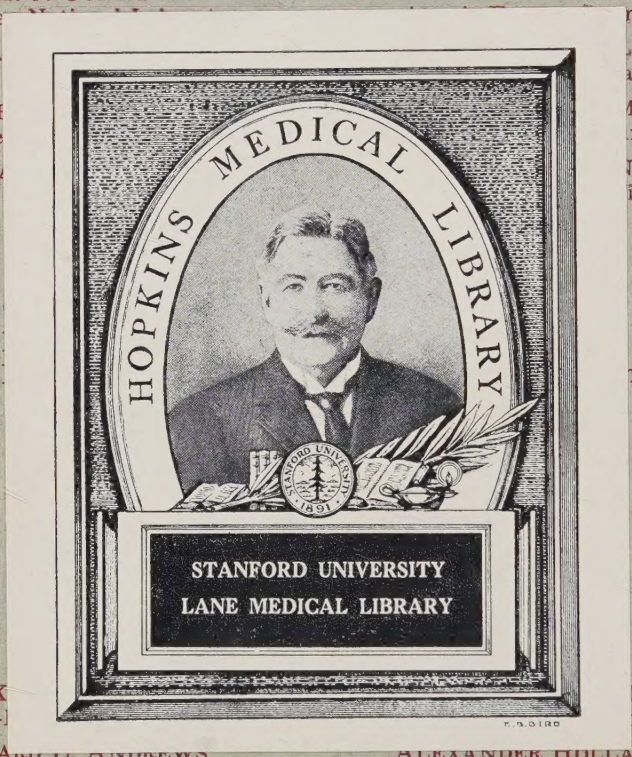

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Nuclear Science Series
Report Number 22

Subcommittee on Radiobiology
Committee on Nuclear Science

RESEARCH IN RADIOLOGY

Proceedings of an Informal Conference

Highland Park, Illinois, May 10-12, 1957

Henry S. Kaplan, Editor

Conference Committee

Henry S. Kaplan, Chairman
Howard J. Curtis
James J. Nickson
Russell H. Morgan
Harvey M. Patt

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FOREWORD

The Committee on Nuclear Science of the National Academy of Sciences-National Research Council has given special attention to those phases of nuclear studies in which two or more of the older, well-established disciplines of scientific investigation have been drawn together. To accomplish this, the Committee has organized a number of subcommittees to provide the wide range of competence needed to consider and deal with these new and rapidly developing areas of science. This is well exemplified in the work of its Subcommittee on Radiobiology, which has originated a series of conferences on the basic mechanisms of radiobiology where physicists and chemists, as well as biologists, meet to exchange information and to test hypotheses of biological actions in the light of the combined experience of the group. This has resulted in free, informal and critical discussions of information available from several specialized fields of scientific study. At the same time, deficiencies in existing data have been revealed and suggestions developed for obtaining the missing information.

The organization of these conferences, arranged to encourage frank and detailed discussion in an informal atmosphere, has been a task of some magnitude. A vital part of the conference is the publication of the proceedings, so that all interested persons may have the benefit of these deliberations. It is obvious, even to a layman in biology who may read these pages, that the vital essence of the fundamental processes are being exposed in the reasonings and arguments which have been recorded. In many instances, it is also apparent that a first tentative approach is being made to the solution of a particular problem. Therefore, it is essential to continued progress that conferences of this kind be encouraged to continue to aid in plotting a course through the myriad of complicated reactions which occur when radiation interacts with living tissues.

The Subcommittee has organized seven conferences. The first was a symposium on radiobiology held at Oberlin College, June 14-18, 1950. It consisted of a series of formal papers and formal discussions; these were published in 1952 ("Symposium on Radiobiology," J. J. Nickson, Ed., John Wiley and Sons, New York, 1952). The second was a highly informal conference held in Highland Park, Illinois, May 31-June 2, 1951, the proceedings of which were not published. The third, fourth, fifth and sixth considered physical and chemical, biochemical, cellular, and mammalian aspects of Basic Mechanisms in Radiobiology; their proceedings have been published as Publication Nos. 305, 367, 450, and 513 of the Academy-Research Council, Nuclear Science Series. No attempt was made to make the discussions comprehensive; rather, topics of current interest were discussed and analyzed.

The topics considered at this conference were suggested by a planning group in which the Subcommittee on Radiobiology was joined by members of

the Committee on Radiology of the Academy-Research Council Division of Medical Sciences and the Radiation Study Section of the National Institutes of Health. The expenses were borne by the Research Grants Division of the United States Public Health Service. It is a pleasure to thank them for this support, not only on behalf of the Subcommittee on Radiobiology, but also of all those who may receive inspiration from reading this publication.

L. F. Curtiss, Chairman
Committee on Nuclear Science

H. J. Curtis, Chairman
Subcommittee on Radiobiology

PREFACE

This volume was prepared from the transcript of an informal conference held at Highland Park, Illinois, May 10-12, 1957. That Conference was the lineal descendant of an earlier series of conferences on "Basic Mechanisms in Radiobiology," reported in four earlier volumes published by the National Academy of Sciences - National Research Council. The 1957 Conference represented an attempt to bridge the gap between experimental radiobiology and clinical radiotherapy. Each major topic was introduced in a relatively formal manner by one of the participants. These introductory remarks soon gave way to general discussion. Since free discussion was the major objective of the Conference, no attempt was made to limit remarks or to make the presentations complete.

The transcripts were subjected to preliminary editing by all participants. Their amended discussions and remarks were then returned to the Editor, who undertook to bring together all of the individually edited sections, to harmonize discrepancies and inconsistencies, and to preserve the spontaneous flavor of the original discussion.

This Conference had two major purposes: to bring together experimental radiobiologists and clinical radiotherapists for mutual exchange of information, attitudes, approaches, and problems; and to explore the feasibility of biometrically designed controlled clinical trials in radiotherapy. It might perhaps have been better if either one of these ambitious goals could have been explored exclusively. Nonetheless, there is some virtue in this arrangement, since the volume encompasses a broad panoramic range from cell physiology and cellular radiobiology at the laboratory level to the introduction of scientific method at the clinical level. Throughout the volume, it is abundantly clear that the problems confronted by the experimentalist and by the clinician, though different in specific detail, are of comparable difficulty and complexity; this realization alone should do much to weld a bond of sympathy and mutual respect between the radiobiologist and the radiotherapist.

Henry S. Kaplan, Editor

BACKGROUND AND PURPOSE OF THE CONFERENCE

Henry S. Kaplan

This conference did not arise de novo. It really has a logical background in the previous series of five yearly conferences on fundamental aspects of radiobiology.

It seems to me, not really having many years behind me to get very much perspective, that the field of radiotherapy stands on the threshold of what may well be a new era. In reviewing its history, I have the impression that many years ago radiobiology had extremely strong roots in clinical radiotherapy. The therapists themselves were the radiobiologists of that time. The work of Schwarz, of Holzknecht, of Regaud, and quite a few others testifies to this. Indeed, many of these ancient contributions are still the foundation on which our practice rests.

I have the impression that in the 1930s, when roentgens came along and attention was diverted to dosimetry and physics, there seemed to be a change in emphasis. Although radiobiology didn't curl up and die, it seemed to depart from departments of clinical radiotherapy and to move over to basic science laboratories presided over by biologists, zoologists, biophysicists, and other fundamental scientists. Radiotherapists for some 20 years now have been operating in a sphere which is rather remote from any day-to-day contact with the affairs of radiobiology. Meanwhile the atomic era came along during the war and gave experimental radiobiology a tremendous shot in the arm and it has since made enormous advances.

It has seemed, therefore, that maybe the time is ripe to bring together some of the people who have been responsible for the advances in radiobiology during and since the war and some of the leaders of radiotherapy in this country to get them acquainted, and to give them an opportunity to air their points of view about a number of aspects of radiobiology that have possible implications for radiotherapy.

This conference is not intended to deal with highly esoteric radiobiology and radiation chemistry. It is oriented toward a subject and should be pertinent to practical problems. The hope is that from this meeting there might come a closer working relationship between therapists and radiobiologists, and perhaps a better appreciation on both sides of the problems and the accomplishments as they stand today.

In addition, clinical radiotherapists have been struggling for years with a good many problems which they have had to approach on an empirical level because of some rather serious handicaps under which they are forced to operate. Definitive solutions to some of these questions would be highly desirable. For this reason also, it seemed that the time might be ripe to bring together a number of minds in an effort to work out the methodology

whereby one could hope to approach some of these questions. I am speaking now of solving questions at the clinical level, of providing conclusive proof that one form of treatment is, for example, superior to another. These are things that all of us who are clinicians are concerned with every day, and I suspect that each of you feel, as I do, that the problem is just too big for any one man to tackle alone.

Moreover, statistical techniques have made some advances, too. It seemed, therefore, desirable to bring into our conference some people with strong backgrounds in statistics and with a working knowledge of how to treat doctors. We hope that after they have been exposed to some of the practical difficulties they may be able to help us to work out methods for attacking clinical problems on a realistic basis.

This is an informal conference. It is true that we have assigned topics for discussion, but the discussants are expected to present them as discussions and not as polished papers which wrap up in nice packages everything that is known or will ever be known about their topics. You may interrupt any discussant whenever you feel like it, either to ask him questions or to challenge and disagree with him. The freer the discussions the more successful this conference will be.

Admittedly, we are trying to bite off a great deal in these sessions and we don't expect the discussions to be exhaustive. It may be that these conferences will seem fruitful enough that you will, as a group, express your feeling that they should be continued, in which case perhaps individual topics might be taken up in more detail at some future time.

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I. FACTORS DETERMINING RADIOSENSITIVITY IN THE CELL

1. Classical Views on Radiosensitivity: Law of Bergonié and Tribondeau, and Relative Sensitivity of Various Normal and Neoplastic Tissues

Titus C. Evans

I have been asked to begin with the so-called law of Bergonié and Tribondeau. I think this is fitting because if this were an opera this would be the overture. For those of you who have children, it is the theme song of this television program. For the politically minded this is the keynote of the convention.

At any rate, the "law" was written in French and it has been interpreted in various ways by Americans. The reference usually given is a very short article.[1] It is surprising how much these Frenchmen said in a very few words. [Ed. The original "law" is: "les rayons X agissent avec d'autant plus d'intensité sur les cellules que l'activité reproductrice de ces cellules est plus grande, que leur devenir karyokinétique est plus long, que leur morphologie et leurs fonctions sont moins définitivement fixeés."] We are all familiar with the principle of radiosensitivity that they set forth. We are not too familiar, most of us, with two other findings that they made.

First, I will just take up the principle. They said three things, as I interpret it. First, they said that the sensitivity of a cell to radiation is proportional to its degree of developmental activity; second, to the duration of this period of mitotic and developmental activity; and, third, to its degree of morphologic and physiologic undifferentiation. In other words, the more undifferentiated physiologically and morphologically, the more active in mitosis, and the longer it stays in this active stage of proliferation, the more sensitive is the tissue. Now it is rather difficult to get around a generalization like this. The words they used are subject to various interpretations and as we get more knowledge I think we can sort of fit it in. If we translate too literally, and generalize too much, of course, we can find objections.

Along with a number of other workers in France and Germany at this time, they found that in the testes, for example, the germinal tissues in the tubules are very sensitive as compared with the interstitial cells. The interstitial cells complete their morphological and functional development early. In the adult, they are not in a state of mitotic or developmental activity and are radioresistant. The interstitial cell hormones continue to be produced after the germinal tissues have been destroyed. In the process of spermatogenesis it seemed to Bergonié and Tribondeau that the spermatogonia that were forming spermatocytes were the ones that were more sensitive, by the criterion they used, which was the disappearance of the cells. They therefore reasoned that the more mature, more differentiated cells were more resistant.

Second, they observed that after a time mitotic activity was resumed, and then they found abnormal figures, chromosomal breaks, sticky chromosomes, and abnormal mitotic figures. They found that in a number of cases they could see where this abnormality had been carried on through daughter cells. Sometimes there were giant cells. They postulated that this mechanism might give rise to the development of tumors after radiation. They also used this as an explanation of the increased radiosensitivity of the tumors as compared to normal tissue. They actually referred to this anomalous situation. Here radiation is used to cure cancer and yet radiation will produce cancer. It was illuminating to me to think about these things that they mentioned so many years ago.

KAPLAN: I am not clear about the second phase of the law that you referred to as having been related to duration of mitotic activity. Are you going to come back to that?

EVANS: No, this is a matter of interpretation, and in reading the article I find that they have not elaborated on it. I take a sympathetic point of view. In other words, I am reading into this that they meant that if, for example, you consider a cell which is still mitotically active but is in the last stage before it completes morphologic differentiation, it would be less sensitive than one that is going to undergo two, three, four, or five divisions before it matures. They didn't explicitly say this. I think they implied it, but I am taking a liberal point of view.

STONE: How do they get around the fact that they say the most primitive are the most sensitive cells and yet after you have killed off a lot of the cells you have regeneration, so that more primitive cells must have been left there in order to bring about that regeneration? Do they have any mechanism to explain that?

EVANS: Again I am taking the sympathetic viewpoint. I would say that they were aware of that, in that they said that even though it was undifferentiated, in this undifferentiated state its reproductive activity and metabolism were low. In other words, a resting embryonic cell would be resistant according to their interpretation, again being optimistic about this. They may not have realized it but they could have. I mean you could read this into their paper.

KOHN: Did they demonstrate regeneration? What were the actual experiments which led them to this? Perhaps it is possible that when they irradiated testes and then examined them in the course of a few days or a few weeks what they saw, of course, was the striking depopulation in the germinal epithelium, but they did not follow the animals long enough to realize that, in fact, regeneration occurred subsequently with the doses that they were giving.

STONE: No, they did follow a long enough time because they saw the giant cells and peculiar cells develop.

EVANS: They might not have followed them for years but long enough to

get this impression and an idea of the way in which radiation produces cancer and they do mention teratologic cells.

FRIEDMAN: I would doubt several things. First, that the spermatogonia are the most sensitive. They are probably the most resistant because they are the only ones that remain behind to reproduce the new spermatocytes and the spermatozoa subsequently. Second, the number of second, third, and nth generation giant cells is infinitely small and gets smaller rapidly, as they are predominantly lethal mutants and eventually disappear. It is my impression that the spermatogonia, which are allegedly in the resting phase, are the most dynamic cells in the testes and at the same time the most radioresistant ones.

CURTIS: Do you know this from actual histological examination, or do you just infer it from the fact that spermatogenesis does go on following a severe dose of radiation?

FRIEDMAN: Not only by histological examination of irradiated testes but also by induction from Donald Charles' graph of the number of sperm available for fertilization after irradiation. It was published in an early Fairchild Atomic Energy report. Charles showed that during the first few weeks after a dose of 300 r, fertilization occurred at a reduced frequency. Then there was a sterile period for approximately one month, and thereafter a regeneration period when apparently normal sperm again became available for fertilization, though at a somewhat reduced rate.

In the January 1949 issue of Radiology, Sturkie and his group described the irradiation of prepubertal roosters' testes with different doses, and there were a number of informative photomicrographs tending to corroborate the radioresistance of the primordial spermatogonia and the extreme radiosensitivity of the mature sperm cells. This is one of the many paradoxical exceptions to one of the laws of Bergonié and Tribondeau.

CURTIS: I think that Bloom also found the same thing during the war.

QUASTLER: There are two kinds of spermatogonia, called A and B, which can be distinguished histologically. The A type spermatogonia are least differentiated and divide infrequently; the B type are somewhat more differentiated and divide rapidly. According to two statements of the law of Bergonié and Tribondeau, the A cells should be more sensitive; according to the third statement, the B cells. In fact, the B cells are much more sensitive than the A cells.

EVANS: Dr. Oakberg had a paper about two years ago in Radiation Research.[2] I remember that he stressed the A and B cells as being two different types of spermatogonia. I should say that Bergonié and Tribondeau were so general in their statements that I could possibly be misinterpreting by saying that they found type B spermatogonia to be the most sensitive. They said the more undifferentiated ones, so I assume this was the type B spermatogonia. But this is again the point brought up by Dr. Stone. The fact that they are undifferentiated would make them more sensitive when they are more mitotically active.

FRIEDMAN: That is not necessarily so. The laws of Bergonié and Tribondeau have been quoted and perpetuated for several generations, but a number of exceptions are accumulating, so that one begins to question their basic validity. For example, there is no proof that the mitotic status of the human tumor cell renders it unusually radiosensitive. The mitotic figures of a radioresistant tumor are extremely radioresistant; the mitotic figures of a radiosensitive tumor are extremely radiosensitive. Consider a radioresistant tumor that requires 8,000 r for its destruction. Its mitotic cells might require 7,500 r for their destruction. On the other hand, in a radiosensitive tumor whose resting cells require 2,500 r for their destruction, the mitotic figures might require 2,200 r for their destruction. Thus the mitotic figures of the first tumor bear no relationship to the mitotic cells of the second tumor. So there is something fundamental in the nature of the cell which chiefly dictates its response to radiation, rather than its phase of mitosis.

EVANS: When you consider their endpoint, their criterion of sensitivity was the disappearance of these cells from the population. With resistant tumors the first cells to disappear are the ones that were active at the time of irradiation, don't you think?

FRIEDMAN: I don't think that has ever been demonstrated. Mitosis ceases but these cells do not necessarily disappear more rapidly.

CURTIS: In plant cells, I think it has been well demonstrated that during the course of mitosis the cell goes through a very marked change in radiosensitivity. The difference is as much as a factor of six in sensitivity between the most sensitive and the most resistant stages of mitosis.

FRIEDMAN: In view of Dr. Kaplan's statement that we are to discuss radiobiology with respect to human tissue, I am trying to stress the weakness of extrapolating data from tissues phylogenetically removed from human tissue.

STONE: In Dr. Friedman's discussion of human tumors I think he has shown that relative sensitivity in any particular tumor goes up and down, but the sensitivity of a dividing tumor cell is greater than the sensitivity of a nondividing cell. One dividing cell is much more sensitive than another dividing cell, but I don't think it is claimed that all dividing cells have the same sensitivity. It is just a relative effect.

FRIEDMAN: Clinical radiotherapy probably received its greatest impetus from Henri Coutard who built the so-called Coutard technique on an erroneous deduction from the work of Regaud, and of Bergonie and Tribondeau. He alleged that squamous epithelial cells undergo mitosis once every seven days, and if you give daily treatments for one month you will be irradiating the cells in their radiosensitive mitotic phase four times during that cycle. The point I am stressing is that the Coutard technique, which is a very successful one, was based on a fallacious interpretation of Bergonie and Tribondeau.

PATT: I don't follow Dr. Friedman's comments about the basic validity of the generalization by Bergonié and Tribondeau. There are, of course, exceptions to it. When he speaks of destruction of the mitotic figures, does this refer to the absence of visible mitosis sometime after irradiation?

FRIEDMAN: My presentation, which follows Dr. Evans', will offer some evidence. I don't know that I am disproving this law. There are a number of loose interpretations of the original laws of Bergonié and Tribondeau. The exceptions are numerous and I wonder how much of the original laws we should save.

EVANS: I would rather not take any more time on this. I want to get to some of the other points that I think are just as important. For those of us who are biologists or physicists, I should like to point out that from the therapy standpoint radiosensitivity and radiocurability are two entirely different things. The important thing is total destruction of the tumor, which may depend primarily upon the amount of energy that you can get in and the amount of damage that you can afford to give the tumor bed, for example. So it is entirely possible, and I am pretty sure it is probable in many cases, that a tumor that requires a tremendous amount of irradiation can still be destroyed more effectively than one which is very sensitive but which spreads everywhere very quickly.

Now I should like to take up the question: what is radiosensitivity? What are the criteria that we use? If radiobiology has any contribution to make to radiotherapy, I think it is the more quantitative aspect that we are trying to develop. We cannot be quantitative until we get some criteria that we can agree on. The physicist likes to have constants and factors that he can put into equations. We have been struggling for many years trying to get these constants. The more we study, the more variables we find, and the more difficult it becomes to understand all of these things completely. But one thing we do agree upon is that we have to set up a criterion and then stay with it. This sometimes gives a false definition, but we have to say, within the limits of our experiment, that we are going to define radiosensitivity in a particular way. The earliest criterion was the appearance of injury. You can see through the microscope that some cells are damaged, and other cells are not damaged. The damaged cells are classed as sensitive, and those that are not damaged are called resistant.

The radiobiologist can extend the use of this criterion to measure the degree of injury produced, as in the reduction in weight of an organ, such as the testis or the spleen. This can be quantitative. The reduction in the number of hair follicles, or the degree of epilation produced, can also be quantitative. The decrease in the mitotic index can be quantitative. But the quantitation that we like the best, the one that we have the best feel for, is to express this difference in sensitivity in terms of the roentgens required to damage one tissue as much as some other tissue. Now if we can say that the difference in sensitivity is equivalent to 500 r and the dose required for one of these effects is 800 r, this gives us a sort of general understanding of the relative amount of difference in sensitivity.

One of the earliest criteria was the median effective dose. We have to decide whether to make the comparison at the dose level which will kill every cell, or at the dose level which will just barely show some effect, or at the dose level which will affect 50 percent of the cells. This last value, the LD_{50}, is the dose which will reduce a given parameter to half of its control value, which is a statistically convenient degree of response. In the literature, you will occasionally find the term LD_{50} with a line and the subscript 30. This means that we are limiting the experiment to 30 days. At the end of 30 days we are going to tabulate the dead animals, and this is the dose which will kill 50 percent of the animals within this period. So we throw the term LD_{50} around quite a bit.

CANTRIL: We don't in therapy.

EVANS: I am talking about radiobiology.

CANTRIL: That is why we have the radiotherapists.

EVANS: Second, we use the latent period as a relative indication of sensitivity. Some tissues respond right away. The one that we usually mention in talking to therapists is the change in the differential count, the relative loss of lymphocytes in the blood. Of course, I realize that some of you are going to say that some of these changes in the blood may be due to migration of cells in and out of the vascular system. This, of course, is a factor at high levels at least. But I think you will agree that lymphocytes appear to be relatively sensitive in that they respond rather soon after a dose of irradiation.

FRIEDMAN: Even that is not necessarily true. Certain lymphocytes are among the radioresistant cells.

PATT: Which lymphocytes are you talking about and where?

BOND: You mentioned that radiocurability and radiosensitivity are different things. Perhaps you will get to this, but how does studying radiosensitivity then help you in assessing radiocurability?

EVANS: I am not proposing that this is going to help the radiotherapists cure cancer, but it is going to help them to understand the mechanisms involved in what they do.

BOND: Then you are talking about a different thing. You are not talking about curability of the cancer itself. You are talking about what you are doing to other tissues surrounding the lesion.

EVANS: I think this is part of the same thing.

FLETCHER: For example, if you take an oral cavity cancer, in order to obtain a high percentage of sterilization you will have to give 6, 000 or 8, 000 r for four to six weeks. That will give a pretty good percentage of

local cures, but then you begin to get complications. If you could do the same thing with four or five thousand r, you would be on safer ground.

KAPLAN: Couldn't one say that the radiosensitivity phase of this discussion pertains to injury? Radiocurability is the result of both injury and repair, and failure to cure is often not due to failure to injure but to persistence of a repair mechanism.

BOND: Then we should modify the statement that sensitivity and curability are one and the same.

EVANS: All I meant to say was that a radiosensitive tumor might not be one that is easily cured. From a therapeutic standpoint, there may be two different endpoints. I wanted to mention that sensitivity is very important to therapy. The sensitivity of the skin might be a limiting factor, or the epithelial lining of the intestine, etc. We have to consider the sensitivity of the supporting tissues. The sequence of tissue sensitivity is usually given in this way: lymphocytes, leukocytes, blood cells, epithelial cells, lining of the intestines, germinal tissue, connective tissue, and then bone, muscle, and nerve. Even bone, muscle, and nerve can be damaged eventually though they are not rapidly growing at the time of the irradiation. Bone, for example, can be called on for regeneration later, as when a tooth is pulled, and then the damage will show. Likewise the brain can be damaged. Maybe the limiting factor here is the radiosensitivity of the cerebral blood supply. I think the therapist takes all of these things into account, maybe subconsciously, but still I think he uses them.

STONE: May we go back to the point you departed from? I am a little lost here. You were talking about the latent period, and then we got off on the sensitivity of lymphocytes. How did we get twisted from the latent period to the sensitivity of lymphocytes?

EVANS: This was to serve as an example. At the other end of the scale, the damage appears only latently, as in the case of bone. In the past we have said it was resistant tissue because it took so long before you saw the effect. If you see the effect soon then you say it is sensitive. The lining of the intestine shows early damage, but may also show early regeneration. Another criterion of sensitivity is the duration of the phase of damage. If a tissue shows radiation damage but quickly recovers, we are not inclined to consider it very radiosensitive.

In summary, our criterion of sensitivity depends upon the techniques used. We start with morphology under the microscope, and at the other end of the scale we evaluate end results in therapy. As we have developed new techniques, especially the use of radioisotopes, they have given us a feeling for finding changes in cytoplasm. The morphologists can see changes in the chromosomes. We think of chromosomes as being the radiosensitive part of the cell. This is because we can readily see the breaks and other effects. By using a C^{14} labeled amino acid, for example, or other labeled compounds, we can find changes in the synthesis of the chromatin material, and the enzymes appear more sensitive as we develop more sensitive criteria. It

seems to boil down to the critical nature of the particular molecule involved. If we have just one or two units that are very critical for the life of the cell, when those are hit we see it. Many, many more molecules of another type may be hit, but we have so many of them and they are so easily replaced that we are not aware of the damage. I think our final criterion must be based on damage that is going to result in the death of the cell.

FLETCHER: I think it would be very befitting now, with the approval of Dr. Evans, if I gave you an interpretation of this paper by Bergonié and Tribondeau. I think your interpretation is not quite right. Let me read it carefully.

EVANS: I am amazed at how much they knew rather than at how little they knew.

KAPLAN: It is becoming apparent that there is not a deadly pall of agreement in the group. Some of the disagreement is perhaps due to difference in language, some of it may be due to our own imperfect understanding of what has been said in the literature, and some of it is due to an imperfect literature. Maybe we can separate out some of these things as we go along.

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2. Patterns of Tumor Regression and Cell Death

Milton Friedman

For over 20 years I have been studying the histologic effects of irradiation on tumors and trying to guide treatment techniques with the aid of information derived from serial biopsies. This has been only partially successful in clinical practice. However, we and others have observed certain consistent phenomena. With this background in mind we continually peruse the radiobiological and the radiation-chemical literature in order to see how many experimental observations resemble ours. In our opinion, certain experimental observations which may not have close application to human cells, tend to be overstressed. It may be helpful to overstress the contradictions in order to sharpen our thinking.

One of our published studies was based on serial biopsy specimens of squamous cell cancers of the upper respiratory tract. We have also studied many types of adenocarcinoma and sarcoma. A number of phenomena were observed, which suggested that there are different ways whereby irradiation affects cancer and tissue. But it also indicated how remote we are from a comprehensive understanding of the mechanism of the effects.

The most important feature is not the physical aspect of radiation or the method of administration, but the susceptibility of the cell.

Acute cell death is the most common radiation effect observed in radiosensitive cells. Its importance has been overlooked because cells undergoing this change rapidly disappear from the tissues and are not seen in later biopsy specimens. Acute cell death may commence within 24 hours after the first irradiation and is most active during the first weeks. The cells melt away by lysis of the nucleus and cytoplasm. The cell membrane, cytoplasm and resting or mitotic nucleus rapidly disappear from view as though they have been etched by acid.

KAPLAN: How did you know they were undifferentiated resting cells? After all, you irradiate them at one point in time. They don't necessarily stay resting for the whole 24 hours.

FRIEDMAN: You may begin to see lysis within 6 to 8 hours after the first irradiation; it becomes intense after 24 or 48 hours and reaches its height by the seventh day. Since the cells in all phases of activity seem to undergo lysis at the same time, we assume that the resting cells as well as the mitotic cells were undergoing acute cell death at the same time.

KOHN: What is the dose?

FRIEDMAN: In almost all of these tumors, with the exceptions that I have mentioned, the average daily tissue dose is approximately 200 to 250 r, and the total dose is approximately 6,000 r in four weeks.

NICKSON: 6,000 r in four weeks is more than 250 r per day.

FRIEDMAN: I didn't say daily administered dose, but the average daily tumor dose, which is what you must consider, because you have to take into account recovery that may go on during Saturday and Sunday when you may not be giving treatment; that is a more significant figure than the daily administered dose which would be 250 r.

NICKSON: This is a matter of arithmetic. 200 r times 20 is not 6,000.

FRIEDMAN: I said 6,000 divided by 30 days or an average daily tumor dose of 200, which is the way it should be stated.

NICKSON: You are counting the days when they are not treated?

FRIEDMAN: Yes, because the nontreatment days are days when recovery goes on.

NICKSON: When do you see acute cell death?

FRIEDMAN: We may see acute cell death within 48 hours after a dose of 400 r in one of the more radiosensitive tumors, such as an undifferentiated squamous cell carcinoma. On the seventh day, there are many areas in the tumor where we can see just stromal structure with no tumor cells.

PATT: What is the rate of growth of this tumor and what would you guess would be the rate at which the cells divide?

FRIEDMAN: I haven't the faintest idea.

PATT: This is quite pertinent to your statement that the resting cells are also destroyed, because these cells, going on with Dr. Kaplan's remarks, may not be as restful as one might think.

FRIEDMAN: Let me put it this way: I may have stated it incorrectly when I said the cells in the resting phase and in mitosis are equally sensitive. What I am trying to say is that when acute cell death occurs in a radiosensitive tumor, the cells just fade away within two days to seven days, almost with equal facility, regardless of the phase of cell division. What you find is denuded stroma, and in some areas you may see cells in various stages of their normal dynamic activity.

PATT: Cells in mitosis at the time of irradiation could be more sensitive than so-called resting cells but this does not mean that the over-all damage is due to injury to such cells alone. If the damage is restricted to those cells that are in obvious mitosis at the time of irradiation, this could not account for the over-all effect that one sees since such cells probably represent only a small fraction of the total.

FRIEDMAN: That is precisely the point I am making. Those who have been studying mitotic figures may be magnifying their importance. We who

look at irradiated tumors through a microscope and observe crude histologic changes, find that mitosis, or the absence of it, is only of partial consequence in the irradiation destruction of a tumor. A few minutes ago I jotted down a list of tumors that are very radiosensitive and yet have little or no mitosis; 1,000 r to 2,500 r may destroy them. One of the most radiosensitive of all tissues is lipoid histiocytosis; a single dose of 250 r is lethal for half these tumors. One sees no mitosis there whatever.

Other radiosensitive lesions with occasional or no mitosis are: seminoma, angioma in infants, angiosarcoma, reticulum cell sarcoma, giant follicular lymphoblastoma, Hodgkin's disease, early Kaposi's sarcoma, many lymphosarcomas and reticulum cell sarcomas, many embryonal tumors such as neuroblastoma and Wilm's tumor, mycosis fungoides, enlarged thymus in infants, and dysgerminoma of the ovary. Another rather large group of tumors which have a small amount of mitosis and yet are moderately radiosensitive are the so-called lymphoepithelioma, a form of highly undifferentiated anaplastic medullary carcinoma of the cervix composed of pale vesicular cells of regular size, and a similar type of lesion arising in the tonsil. A giant cell tumor, when in the benign, nonmitotic stage, is relatively radiosensitive. When it grows actively and has a lot of mitosis, it is very radioresistant. Another similar paradoxical lesion is Hodgkin's sarcoma, wherein the acquisition of mitotic figures is frequently associated with increased radioresistance.

Contrariwise there are a lot of tumors with many mitotic figures that are very radioresistant: osteogenic sarcoma, adenocarcinoma, bizarre pleomorphic squamous cell carcinoma, melanoma, certain connective tissue sarcomas, and many other cancers which may have a lot of mitotic figures and be radioresistant. Therefore, based on clinical radiotherapeutic observations and crude though careful histologic observation of serial biopsies after irradiation, mitosis has only slight importance in the radiosensitivity of many tumors. It is the inherent characteristic of that tumor about whose radiosensitivity we know practically nothing.

LAMPE: When was the biopsy taken?

FRIEDMAN: On the seventh day after treatment, when the tumor dose would be about 1,400 to 1,600 r.

LAMPE: This is carcinoma of what?

FRIEDMAN: Most of these are squamous cell carcinomas of the tongue, pharynx, or tonsil.

There is still another area showing acute cell death and lysis. I cannot demonstrate it here but we saw cells seemingly frozen in a stage of mitosis and disappearing by this lytic, almost chemically etched phenomenon.

Now to prove once again that mitosis is not necessarily the most radiosensitive phase, a squamous cell carcinoma (grade 3) after two treatments totalling 500 r was developed. A biopsy was taken on the third day. The

polyhedral cells, which are the resting cells, are the first to show cytoplasmic swelling, whereas elsewhere in the tumor mitosis has not been interfered with. This finding is infrequent but is occasionally seen. A possible explanation is that in the polyhedral cell the nucleus is not dynamically active, but the cytoplasm is functionally active. It seems that the dynamically active portion of the cell is radiosensitive, i.e., the nucleus, if the cell is actively undergoing mitosis; or the cytoplasm, if the cell is functionally active. Other examples of the latter are the thyroid parenchymal cell, which is resistant when normally active, and radiosensitive when hyperactive; or the pituitary gland whose chromophil cells are resistant when normal, and radiosensitive when active, as in acidophilic adenoma.

LAMPE: Don't you occasionally see changes like that, that are spontaneous in the tumor without treatment?

FRIEDMAN: Yes.

LAMPE: How do you know that this isn't just the section that you have picked out and that it isn't showing some degenerative changes that have nothing to do with radiation?

FRIEDMAN: I tend to be an enthusiastic microscopist. Dr. John W. Hall, who died a month ago, was an extremely conservative man who held me down. In this study[1] we critically evaluated the possibility of whether this could have occurred spontaneously. We looked exhaustively in this tumor before treatment to see manifestations of such spontaneous change. This is our estimation of what we think happened to these tissues as a result of irradiation. We could be wrong but I doubt it.

KAPLAN: It seems to me that you have to consider alternative interpretations. Maybe Dr. Pomerat is going to get into this question when he shows us some radiation effects on cells in tissue culture. There is an increasing amount of evidence to suggest that when a cell does this sort of thing, when we get giant cells in irradiated cultures, for example, we are actually seeing a manifestation of injury to the mitotic process. The cell is unable to undergo mitosis, but it can still go on with its housekeeping, such as the manufacture of protein and cytoplasm. These cells that you have shown us could just as well be interpreted as cells that are out of phase in this sense. Normally when cells build a certain amount of new protein they then get some kind of signal to divide. The irradiated cell can't divide but it can still keep on making protein. If you interpret what you have shown us in that light, mitotic injury remains a major aspect of the response.

FRIEDMAN: I have other photomicrographs in all of which the first change in the tumor cells was cytoplasmic swelling without any effect on the nuclei.

Now remember that you are asking me to look at radiobiologic experimentation based on nonhuman material, and I think I am speaking for other clinical radiotherapists when I suggest that the radiobiologists look at human tissue and try to reorient their observations with what is seen in such tissue.

EVANS: We have seen things like this in animal tumors. This isn't new or startling, but the interpretation is a little startling.

FRIEDMAN: I am relating this not to indicate that it is really startling, but to bring out the numerous exceptions to accepted and possibly questionable laws and opinions, namely, that the mitotic figures are always the most radiosensitive elements of all. I frankly don't believe it.

BOND: You are downgrading mitosis. What do you believe then is the basis for fractionation? Why do you think that fractionation differentially affects the tumor as compared with the normal tissue through which the beam passes?

FRIEDMAN: Only one of the cellular phenomena produced by radiation is that which occurs in the mitotic figures. I would venture to guess that there are at least 150 other phenomena about which we know little or nothing, which participate in the recovery process. When the daily dose is large and the tissue is radiosensitive the overwhelming effect is destruction. More often in the irradiation of human cancer, the effective daily dose is not large, the tumor is not very radiosensitive, and the tumor not only tends to resist irradiation but, after a short time, recovers from irradiation effects. Often there are alternations between destruction and recovery resulting in a cyclic phenomenon that has been termed periodicity.

BOND: Do you have evidence of this?

FRIEDMAN: A little evidence here and there concerning the different types of recovery phenomena and some speculations. I have listed about 50 phenomena, suggesting many different mechanisms whereby protoplasmic material may respond to irradiation. The effect on mitosis is one of many.

KOHN: Would it be fair to say that what your research shows, without interpretation, is that the mitotic index of a tissue is not directly correlated with the sensitivity of the tissue to X-ray, when that tissue is also considered in relation to other kinds of tissue?

FRIEDMAN: That is decidedly so, and to paraphrase it, mitosis as an index of response to irradiation is not very important.

KOHN: I think that is going too far beyond what most people would claim, because it seems to me that most of the biological experiments have taken pains to compare a particular tissue with its own behavior at various stages of the mitotic cycle. The people doing these basic experiments are attempting to show, for a particular tissue under controlled conditions, that as cells pass through their cycle of division there are some phases that are much more sensitive than other phases. Certainly I think we can all take it as accepted that this has been shown.

FRIEDMAN: I agree with all this. I said in the beginning that this is true, but it is not at all important in the radiotherapy of cancer, as I indicated a moment ago when I said that I have a list of tumors having marked mitotic activity and yet little or no radiosensitivity.

KOHN: So you would say that taking one of these cells, if we could do an experiment in which we irradiated it at various times in its cycle of division, we would find, starting early in prophase and going through to the end of telophase, that this period is the least sensitive part of this cell's life history?

EVANS: Don't you think that mitotic activity is related to the malignancy of a tumor? I mean as to whether it is harmful or not, and if it is not growing it is not very harmful? Sarcoma must be growing pretty fast.

FRIEDMAN: Maybe I have it on my list. An example of a highly malignant tumor of low mitotic activity is the melanoma, which is a deadly killer.

EVANS: What kills the person?

FRIEDMAN: That is what we don't know.

DICKSON: Melanoma can be of very high mitotic activity.

FRIEDMAN: It can be of very low mitotic activity also. Let me phrase it this way: melanoma is one of the deadly killers. It is generally radioresistant, and it does not matter whether it has a great deal of mitotic activity or little mitotic activity, in general it is still radioresistant.

DICKSON: But it varies in its killing powers with its mitotic activity.

FRIEDMAN: Yes, within a certain range.

PATT: I would like to go back to one point that I think is very important, namely the meaning of the mitotic index. The mitotic index as ordinarily defined is an ambiguous quantity, because one tacitly assumes that all of the cells in the particular population that is counted are capable of dividing and are in fact dividing at about the same rate. What is really needed is a mitotic index expressed in terms of the cells in the population that are actually doing the dividing.

FRIEDMAN: If you get a group of radiobiologists who have spent years counting mitotic figures and they come together in a meeting, of course, mitotic figures are going to be important.

PATT: I am merely making the point that the terms "mitotic index" and "mitotic activity" have a variable meaning.

The second point I should like to make is that animal tissues that are ordinarily thought of as radiosensitive may show more mitotic figures several days after irradiation than relatively resistant tissues. Let's consider skin or the adrenals in contrast to intestinal epithelium. Mitoses come back sooner in intestinal epithelium than they do in the relatively more resistant skin and adrenals, possibly because of a shorter intermitotic time. I think this is an important consideration in the interpretation that you are trying to make. The fact that you look at tissues a few days after irradiation and happen to see mitosis coming back does not necessarily indicate that sensitivity is not related to mitotic activity.

FRIEDMAN: It is not based on just one observation.

PATT: I am talking about the interpretation, not the number of observations. I would not consider mitosis as the only predisposing factor in radiosensitivity. Certainly there are many factors in sensitivity. I do not hold any brief for mitosis alone, but I think we must recognize that it is an important factor notwithstanding the exceptions.

NICKSON: You have said that the mitotic index is not reliable. What do you suggest as a reliable index? It seems to me that taking away the normal criterion for mitotic activity without substituting something else leaves us rather hanging in the breeze.

PATT: What I am saying is that you can look at two tissues and find in each a mitotic index of 1 percent: one out of every 100 cells is a mitotic figure. In the case of one tissue we might assume that all of the cells are capable of dividing. In the other tissue perhaps only 10 percent of the cells are capable of dividing. Hence an expression of the true mitotic activity in the latter instance would be 10 percent, not 1 percent.

NICKSON: It seems to me that this is a most important fine point.

PATT: Yes, it is exceedingly important.

NICKSON: But you destroy the mitotic index as a criterion.

PATT: I am not destroying it. I am pointing out that it is a very ambiguous criterion and one should try to define just what it represents.

NICKSON: Practically you have destroyed its validity as a criterion for evaluation or am I overstressing it?

PATT: I am just raising this point of caution regarding definition and interpretation. Take bone marrow for example. If you make a count of the cells in bone marrow and you include in your count cells that you know are not capable of dividing, such as the polymorphonuclear cells or the band cells, obviously you are diluting out your index of mitotic activity.

KAPLAN: It seems to me that we are talking about two different things. One is that during the mitotic cycle of any given class of cells there will be a variation in radiosensitivity, which for some tissues in which this can be studied sequentially turns out to be maximal in the period immediately before cell division begins, that is just before prophase. Thus there is a factor relating to mitosis.

However, Dr. Friedman also has a point in that for different classes of tumors, all of which grow and all of which divide, there are vast differences in radiosensitivity which have to do with inherent differences in cell type and which we do not understand. These differences, from the standpoint of the therapist, are of considerably greater importance; but I do believe that you are pushing your evidence too far and confounding it with the other aspect.

FRIEDMAN: I think it is necessary to do so, and I think it is also useful to suggest to the radiobiologists that they stop counting mitotic figures and find out what these inherent characteristics of the tumor are which contribute to its radiosensitivity.

KLIGERMAN: The mitotic index is just one index of radiosensitivity and it does not mean that cells cannot be destroyed even if the cells are in the so-called resting stage. There is clinical evidence for that, and I think it is this clinical evidence that is confusing a good biological standard of sensitivity, the mitotic index. For instance, we can treat carcinoma of the skin in seven minutes. We can give that tissue, we will say, 2,000 or 2,200 r. This can be delivered in seven minutes or less, and there may be some cells in this tumor (there must be because we can cure 95 percent of these tumors by this method) which, indeed, are true resting cells and they are destroyed. The fact that cells can be destroyed in the resting stage does not mean that the mitotic index isn't an indication, a standard by which to measure radiation sensitivity.

FRIEDMAN: In my experience it is not, and I have shown you a number of radiosensitive tumors which have little or no mitosis in them. I will grant only that if a cell is in mitosis you can destroy it with perhaps 10 or 15 percent less dose than if it is in the resting phase.

STONE: I think we can all agree with what Dr. Friedman has said, that you can kill cells that are not in mitosis. That is about the essence of what he has proven and the rest of it is his interpretation.

FRIEDMAN: One of the less important mechanisms of radiation effect on squamous epithelium and squamous cell carcinoma is a most interesting one. I think it is a purely cytoplasmic phenomenon, namely, the acceleration of keratinization. Let's look at the various manifestations of this phenomenon.

In a case in which the breast was infiltrated by carcinoma, I was able to get serial biopsies of skin as well as tumor. I think about 15 biopsies were taken from this patient in a month. On the 18th day there was a partly red erythema, also there was a brownish discoloration which was interesting because it was due to accelerated cytoplasmic keratinization. On the same day, we observed thickening of the stratum corneum. There was noticeable cytoplasmic swelling, and at this time there appeared to be a mitotic figure. There again was an acceleration of keratinization. On the 24th day we observed additional layers of epidermis undergoing keratinization. As long as a basal epithelial cell was viable, it seemed not to separate itself off from the dermis. Now on the 38th day there was almost complete denudation. Most of the destructive effect had been due to the accelerated keratinization of the skin and the thickening of the stratum corneum to the point where the entire epidermis became cornified and was desquamated.

Now, let's discuss accelerated keratinization of normal mucosa. Another patient had a cancer of the tonsil. We saw normal mucosa of the edentulous upper alveolar ridge, hard palate and retromolar triangle. All

these tissues received the same dose of irradiation. We noted that some parts of the mucous membrane showed a whitish leukopiakic membrane of recent origin. Irradiation had accelerated the manufacture of eleidin, which is white, in the normal mucosa. However, of particular interest was the absence of the white membrane in the retromolar fossa. This region revealed a nonkeratinizing type of mucosa.

In another case of carcinoma of the tonsil following a tissue dose of 3,000 r in 14 days, there was pseudo-diphtheritic membrane of the soft palate. This is true second-degree epithelitis with denudation of the mucosa. The hard palate mucosa, on the other hand, underwent a thickening of the keratin layers, forming a radiation-induced leukoplakic membrane.

In the case of a girl with a lymphosarcoma of the rectum, one of the treatment portals was directed through the anus and perineum. As keratinization was accelerated the keratohyalin of the skin took on a brownish color, whereas the anal skin behaved like a mucous membrane and was discolored white. A few weeks later when the reaction had healed, dermal pigmentation, which is still a different type of brownish discoloration, was left in the skin of the buttock, whereas the anal skin had no melanin pigmentation and appeared very pale.

So much for radiation-accelerated keratinization of normal skin and normal mucous membrane. An inherent characteristic of certain tumors, namely, spontaneous excessive keratinization, manifests itself as parakeratosis. In one such tumor the cells changed from undifferentiated carcinoma, rapidly proceeded through parakeratosis to become mummified in keratin and desquamate off. Wherever you see this phenomenon of dynamic parakeratinization in the tumor before treatment, you know that it will be extremely radiosensitive and that the dominant mechanism of radiation effect will be through accelerated keratinization. It does not occur often but you can occasionally detect it beforehand.

Sometimes in a tumor, before treatment, there is such marked spontaneous keratinization that you will see foreign body giant cells (this is before irradiation has started). This kind of tumor will be radiosensitive, even though according to Broder's classification it is differentiated. After irradiation of squamous cell carcinoma, there was a tumor cord and there was an island of the tumor in cross section. Roughly I would guess that in this tumor keratinization occurred in about 15 percent of the cells. This was found in a biopsy before treatment. On the third day after two treatments of 250 r tumor dose, about 50 percent of the cells were keratinized. One week later, which would be about the ninth day of treatment, most of the tumor was destroyed. The external part was desquamated off. Tumor cells in the interior had become completely keratinized, and there were phagocytic multinuclear foreign body giant cells. This had nothing to do with mitosis.

KAPLAN: Please don't raise that red flag.

FRIEDMAN: I think it is a very important one.

KAPLAN: We don't agree with you.

FRIEDMAN: In a teratoma of the testes, composed of many types of structures, you can also see the phenomenon of keratinized tumor cells and phagocytes.

Picture all of these numerous phenomena going on at the same time. In a particular cell, for example, keratinization already had started under the cell membrane and froze any possible active metabolic effect. In another cell there was cytoplasmic swelling and vacuolation, which is a different mechanism of radiation effect, and in another there was radiation effect on the nucleus, but time does not permit further discussion of these.

KAPLAN: Again we have this unanimity. It is very heartening.

FLETCHER: I see that Dr. Zirkle is going to tackle this matter of the mitotic stage, and I think it would be fitting, in memory of Bergonié and Tribondeau, to translate these laws. "X-rays are more effective on cells with greater reproductive activity." That is one. "There are also cells which have a longer dividing or mitotic future." What this means is that two types of cells are reproducing fast, but one type has, let us say, 1,000 mitoses to go before it reaches its final stage, and the other has, say only 100. Those which have 1,000 mitoses are more radiosensitive and have a longer period to go before they eventually get to the final stage. When you have reached that stage the cell does not divide any more, because it is mature. The number of divisions before you reach the final mature stage is obviously what they mean by the term "mitotic future."

QUASTLER: Is it obvious? I don't want to dispute your French.

FLETCHER: The dividing future is longer. That is, if you take the sequence from the original spermatogonia to the spermatozoa, the sensitivity of the various spermatocytes is greater the farther they are from the final spermatozoal stage. Bergonie and Tribondeau have no evidence--well, the evidence they have is probably what they thought was the relative disappearance of these various spermatocytes.

The third part of the law states that sensitivity is greater in cells when their morphology and their functions are less fixed. Here, of course, they were mostly struck by the difference between the spermatogenic cells at various levels and the interstitial cells, which are very well fixed in their function.

There is no reference at all in the paper to a greater sensitivity depending on the phase of mitosis or on mitosis versus the resting phase.

EVANS: Not in just that way, but they do talk about tumor sensitivity and irradiation at different times being best.

FLETCHER: Just a minute. "Of course, tumor cells are known for having a great mitotic future and, therefore, this is why they are more

sensitive than normal tissue. This is a conclusion which is drawn logically and not experimentally."

They also refer to reports that irradiation can produce skin cancer, and they relate this to their own observations in irradiated testes of abnormal cells with atypical mitoses. They make, by the way, a very profound statement, that these cells have undergone mutation and that this is the first step in the evolution of cancer. When they suggest using small fractionated doses, they do not mean fractionation in order to enhance sensitivity but to avoid the possibility of producing cancer.

From the standpoint of radiotherapeutic practice the thing I get from these statements is to avoid these atypical mitoses that produce many mutants, and the method of using small and repeated doses is better from that standpoint.

There is no statement that the mitotic phase is the phase of sensitivity.

EVANS: I didn't mean to say that.

KAPLAN: He didn't say that.

J. R. ANDREWS: I believe the first point made here, Dr. Fletcher, was essentially that the reproducing portion of a given population of cells is the most radiosensitive. Is that assumption correct?

FLETCHER: No.

KAPLAN: I should like to suggest that this is out of order, since we have been over this ground. We have nailed down the fact that we do not understand their "law" and that we all disagree with it.

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3. Relation of Cell Injury to Mitotic Stage, Ploidy, and Site of Ionization

Raymond E. Zirkle

There are several papers which seem to have a fairly direct bearing on the question of whether the ploidy of a given type of cell has anything to do with its radiosensitivity, in things as diverse as barley seedlings, Drosophilia embryos, Tradescantia chromosome aberrations, yeast cells, and so on. Just about all the answers possible have been obtained. In barley seedlings Müntzing measured rates of growth after irradiation with X-rays and found that tetraploid seedlings are more resistant than diploid, which would indicate the greater the ploidy the more the resistance.

In Drosophila embryos, Lamy and Muller some years ago had the opportunity to study triploid versus diploid embryos, and they came out with the same sensitivity in both. This was based on the mortality of the embryos at a somewhat distant date after irradiation.

In the wasp Habrobracon, which has been used so much by the Whitings and their school in various radiobiological experiments, haploid males can be compared against diploid males or against diploid females. In these cases the haploid male turns out to be more sensitive to X-rays than either of the diploid sexes, which would indicate again that the greater the ploidy the more resistance.

Quite a bit has been written on Tradescantia. There is one particular experiment which is based on a single flower, but it looks like a good experiment nevertheless. Nobody knows how this situation arose, but Conger and Johnston found a single flower in which they observed both haploid and diploid microspores in approximately equal number. It so happened that this flower had been X-rayed at a known time before this observation was made, that is, before the flower was fixed and smears made for counts, and they were able to study chromosomal exchanges in the haploid and diploid microspores in this very closely related material. The exchanges per chromosome were the same for both ploidies.

This brings up a point. You have to be careful how you express your result. If they had expressed it as exchanges per cell, then they would have had the diploid twice as sensitive as the haploid or only one-half as resistant and this is in contradiction to the observations on barley seedlings, etc., that I mentioned before. I think at this point one can conclude that the effect of ploidy as observed upon cell populations depends on the species you are dealing with, the effect that you are actually scoring, and probably on the way in which you express your data.

In yeast, which I suppose has been investigated from the standpoint of ploidy more than all the other objects put together, I think all of these difficulties point up. I should like to give you a little summary of some of the

material which has been accumulated during the last eight years on the effect of ploidy on yeast cells. It has not been very long that yeast cells of different ploidy have been available and, as a matter of fact, the X-ray experiments were performed shortly afterward.

The first were by Latarjet and Ephrussi.[1] They used haploid and diploid yeast cells. Here it is necessary to state as explicitly as we can just what it was they scored in trying to do a quantitative experiment. They studied essentially the inhibition of the irradiated cell's ability to produce progeny. They did this in two ways. They determined the number of cells which were unable to carry out even one cell division after irradiation. They did another experiment in which they determined the number that could complete one division and then balked when the second was attempted. Moreover they studied inhibition of macroscopic colony formation. In other words, they plated out these irradiated cells and observed the reduction in the number of colonies countable by the naked eye. That last, it turns out, seems to be the most reproducible method, as it has been used by most people who have studied this matter since.

What did Latarjet and Ephrussi find? If we plot the logarithm of the survivors as the ordinate and the dose as abscissa, they found that the haploid gives an exponential curve. In other words, it shows up as a straight line when the ordinates are plotted in logarithms.

On the contrary, the diploid gave a sigmoid curve. It does not matter at what level you want to draw a line and compare the corresponding effective doses, there is a factor of several in sensitivity between these two strains of yeast. I should emphasize that these are genetically-related strains of yeast. Presumably, in other words, the genes in this one are essentially the same as in this one, except that this has a double dose of genes and this has only a single dose. So there is a very clear-cut case of the influence of ploidy when one studies inhibition of ability of cell to produce progeny. Practically everybody else who has studied this since then has obtained essentially this same result with haploid and diploid yeast. Sometimes the spacing between the curves is greater than I have drawn here and sometimes less, depending upon the particular strain of yeast. I don't mean strain with respect to ploidy but with respect to a lot of other genetic factors, most of them as yet undetermined.

Suppose you use yeast of another ploidy, say triploids and tetraploids. Would you expect to come out with curves indicating still less sensitivity? Tobias and I[2] had a theory to explain this effect of ploidy, and we didn't have any tetraploids and triploids to test experimentally, so we just assumed that we would get curves of less and less slope. A couple of years after we had come out with this theory, Mortimer, a student of Tobias', actually got strains of diploids and tetraploids by proper matings. They were related to the diploids and the haploids, and they didn't respond according to prediction at all. Quantitatively the triploid curve came down there and the tetraploid somewhere in here. So here is a seemingly very complex situation with respect to ploidy, based on inhibition of colony counts.

It would probably taken an hour to tell you where Tobias and I went

wrong. Our theory involved the notion that to inactivate a haploid yeast cell so it could not produce a given number of progeny we had to affect one unit of some sort in the haploid cell, and this one unit might be one of quite a few different kinds of units. The result would be this type of curve. By assuming that you had the same kind of units in the diploid cell as in the haploid but just twice as many of each kind, you could arrive at a pretty fair explanation of this diploid curve also. However, we assumed that in order to keep the cell from dividing you had to knock out both of some one kind of these hypothetical units. That was our recessive model for explaining the difference between haploid and diploid survival curves.

According to the recessive model, to knock out the triploids, you would have to knock out all three units at the same time and for the tetraploids you would have to get all four of a given kind of unit and that would put their curves out here someplace. That didn't happen at all. Just the opposite. However, if along with these recessive changes that Tobias and I hypothecated you are also getting some dominant ones, such that you will have to affect only one unit regardless of how many are present in the cell, then you can see that the situation is reversed. For a given type of unit in a haploid cell you have only one chance at it, but in the diploid cell all you have to do is to get one of two. Your chances are double there, and that would be a more effective mechanism. In the triploid you would have a greater chance for a dominant effect, and in the tetraploid you would have still more. Why this order? The recessive effect is going on, too, apparently. At least that is the easiest explanation. In the haploid, most of the response is due to the recessive effect, as a matter of fact. In the diploid there is a substantial contribution from both of them, but as the ploidy goes up, the dominant comes to dominate, if I may use the term, and the recessive mechanism gets to be correspondingly more unlikely because you have to get all three units, say, in the triploid.

Now I should like to bring out one more aspect of Mortimer's work. Remember that this information is all based on inhibition of cell division. Mortimer[3] is the daddy of the idea of the dominant model for accounting for this difference in "sensitivity" and he has done a lot of experiments to test for dominant responses. He can irradiate a haploid cell, for instance, and then mate it with another haploid cell. If a dominant mechanism is operating, then the zygote formed from that mating should be inviable, whereas if we are dealing with an entirely recessive model, then, of course, the zygote should be viable because it has one good complement of genes. That essentially is the nature of the experiment. There are ramifications to it which are not necessary to go into here. When he does that experiment and scores dominant lethals, as we call them, he gets curves with the haploids and diploids in which the response is backward, as you would expect, because you have more chances of hitting something in the diploid. Specifically you draw a line across here for comparison and drop down to the dose axis. You can see that the doses are roughly in the ratio of two to one, which fits fairly nicely, whereas over here when we are dealing with inhibition of cell division the ratio is more than two to one. So you get different quantitative answers depending upon the actual effect you score. That is what I said a short time ago and I have gone through this, using Mortimer's rather accurate data, to bring out just that point.

Dr. Kaplan also asked me to say something about radiosensitivity and the stage of mitosis, and I will say categorically that one can say the same thing there as about ploidy. That is, you can get different answers depending on the species of cell you are studying, the thing you are scoring, and I imagine a whole list of other factors. In a paper published in 1951, Arnold Sparrow[4] drew up a table in which he summarized, I suspect, almost all of the previous literature, going back to 1926, on microorganisms, chicks, grasshoppers, Tradescantia, sea urchins, frog eggs, onion root tips, etc. Various investigators, working on these different types of material, probably with different techniques, and certainly observing and scoring different effects, came up with different answers. That is, you can find any stage of mitosis, including the interphase, that you want to find here in this column of stage of high sensitivity. I didn't check carefully to see if they are all also in the stage of low sensitivity, but most of them are. So apparently sensitivity varies all over the mitotic cycle, depending on the biological factors, the most obvious of which are the species and the effect that you are studying.

If nobody wants to argue about that particular point I think I will leave it just as it is. There is no question, though, that when you study a given type of cell there are variations in the sensitivity through the mitotic cycle. Sparrow cites some extreme variations; I think one is a factor of 60, and another is a factor of 15. There is mostly a factor of about 5 between the most sensitive and the least sensitive stage.

WOOD: Is the stage always the same, or relatively the same, at which they are sensitive?

ZIRKLE: There seems to be a little tendency for the very high values to occur in metaphase or late prophase.

WOOD: Yeast is six times as resistant in the dividing cell as in the normal cell, so there is an extreme range of resistance.

ZIRKLE: That is right. I had forgotten that business of the yeast bud, that in the dividing state it is more resistant rather than more sensitive. Actually there are a few cases here in Sparrow's chart where the interphase is the most sensitive, too.

Rather than to belabor these factors affecting sensitivity, I thought I would talk a little about ways of producing abnormal chromosome sets. I don't think I will spend any time on the production of polyploidy, abnormally high ploidy, but on aneuploidy where the chromosome balance is upset. That is, where there is an extra chromosome or an extra chromosome fragment or maybe an extra group of chromosomes or corresponding deficiencies. There are a number of ways in which such effects can be obtained. I don't know that I know the complete list, but one of the commonest and the longest known effects is what is known as stickiness. When I used this term two years ago at one of these conferences, the chemists present promptly jumped down my throat and wanted to know what I meant by stickiness. The cytologists present said, "There is nothing to it. They just stick together and that

is chromosome stickiness," and that is about what we ended up with. I can demonstrate this for you and let you see how it develops in a single newt heart cell in tissue culture, using time lapse photography with a speedup of about 60. Preparatory to this I would like to show a normal mitosis in such a cell, so that you can see the contrast after a similar cell has been x-irradiated.

(Movie) This is a late prophase and you can see the nucleus. The nuclear membrane broke down just as I spoke. The entire field, you see, is something like 45 by 60 μ in diameter. These are quite large cells, as you will notice. They are two or three times the linear dimension of human cells. You can see one end of the spindle here. You can also see the centriole in the centrosome when it happens to be in focus. The other end of the spindle is up here. This clear zone does not have any cytoplasmic granules in it. You will notice the chromosomes are aggregating on the equator of the spindle. They are all hairpin shape.

The kinetochores are on the surface of the equator of the spindle in this particular type of cell. The kinetochore, or the centromere, if you prefer the term, of this one is nuzzled up against a centrosome. You will see the little centriole. There is an attraction that apparently is determinative of the behavior of such a chromosome for a while, but normally chromosomes behave that way only temporarily; they always move on and join the others on the metaphase plate before the anaphase occurs. That one did. There goes the anaphase; notice how promptly the normal chromosomes move apart. There is what we call a late peeler. That is, two chromatids of the same chromosome that are going in the opposite direction peel off a little later than the others. Here is the cell constriction coming through here and chopping the cell body into two cells, and here is a nucleus reconstructing out of one group of chromosomes and the other one is way up here doing the same thing. (End movie.)

Now with that sequence fresh in your minds I would like to show you a similar cell which was given 350 r of 200 kv X-rays. As a matter of fact, the whole culture was given that dose. We had to do that in this particular experiment, but I don't think there was enough of an indirect effect to influence this response. As a matter of fact, we have bombarded single cells with protons and left the rest of the culture unbombarded, and have obtained similar results.

(Movie) There will come a gap in the film presently. That is time out for irradiation. Here again is the prophase. This film is a little darker than the other. That was the time out for bombardment, and you see the cell progress during the two or three minutes it took to deliver the dose and to get it back under the microscope. Now you can see the spindle forming here. Here is one end. It is obscured somewhat by the chromosomes, and you can see it elongating there. That is the way the spindle forms, incidentally, in these cells. After you had looked at a few thousand normal cells you would certainly say that this cell is sick; that is, the wiggling around of the chromosome is much reduced over that of the normal cells you saw a minute ago. I don't think the metaphase was greatly prolonged in this cell,

although it was changed to another microscope. We ran out of film and had to make a quick shift. The metaphase can be prolonged by irradiation, but duration of metaphase in these cells is normally quite variable.

There is an attempt at anaphase. Notice that many of these chromatids are stuck together. The two groups as a whole can't get apart, and while they are still struggling to get apart reconstruction is beginning. If it were not for the cell constriction you would get a long single nucleus reconstructing there. What we are going to get is a dumbbell-shaped nucleus, because the cell constriction comes in and does not cut completely across this mass of chromatin; instead there is a little gap. Here is a nucleolus reconstructing. If you only saw this much you would say that it is a perfectly good-looking resting nucleus or a nucleus in late reconstruction. (End movie.)

Let's talk about the mass of chromatin materials. They must be grossly different in genetic constitution because of the randomness with which a good share of the chromatin got distributed. Another thing can happen. Instead of coming right across the middle of this group of adherent chromosomes the constriction may slide off to one side or the other. The result is that there is complete cell division, one cell getting no chromosomes whatever and the other getting both sets and, therefore, ending up as a tetraploid, since these normal cells are diploids. That type of phenomenon, of course, has been seen in stained preparations since 1905, or thereabouts, in plant and animal cells. This shows how it develops. You can get this effect pretty frequently with 200 r or even with 100 r. With 200 r I think you get it about half the time, and with 350 r you get it every time, but there are differences in detail, differences in the cell constriction, and so on.

KOHN: When you say that with 350 r you get this every time, do you mean in every nucleus in the field?

ZIRKLE: Usually in the newt the mitoses are so few and so far between that you will have just one mitotic cell in a given field. What I meant is that at 350 r out of 100 experiments you will get it 99 times at least. In our case, each experiment involves only one cell. With 200 r, as I said, such aberrations will happen about half of the time, and with 100 r you will get them a few percent of the time, so that you can get these aberrations with relatively low doses.

What is the effect of the stage of mitosis here? Usually when we irradiate cells that are already overtly in mitosis, we cannot find very early prophases but sometimes we do. We can get all the metaphases and telophases we want. Of course, telophases don't help us with this stickiness problem simply because the chromosomes are already separated, but with respect to the prophase and the metaphase there isn't much difference in sensitivity. It takes a long time to do 100 of these, of course, so that our data are not terribly accurate on this point, but there certainly isn't much difference between the prophase and the metaphase until about 10 minutes before anaphase and then the resistance goes up by a factor of at least 2 and probably greater. In other words, whereas 350 r in an early metaphase will produce this effect almost every time, if you irradiate within 10 minutes of the anaphase you

won't get more than 20 percent of the cells showing such an aberration. So there is a big change in the "sensitivity" at that time.

Now can you get this by irradiating an interphase? We haven't done that directly because the interphase is very long, several days in these cells, and the thing that Dr. Patt was talking about awhile ago comes strongly into play here. We don't know what fraction of these cells in the tissue culture have mitotic potentiality. We don't know whether 10 percent of them are destined to undergo mitosis or whether it is 1 percent or some other fraction. So the practical result is that we don't try to work on interphase cells, because we have a strong chance of selecting and watching one that is not destined to go into mitosis at all. Instead we irradiated the whole culture. Then we kept those cultures, after studying the one cell we were particularly interested in, and observed them every day for the next week or so, and we still got this picture of the sticky chromosomes, and the dumbbell-shaped nucleus quite frequently. The cells producing those had to be in interphase at the time of irradiation. There is no other way of interpreting such a result, in view of the time relations. So the answer is that qualitatively you can produce this effect by irradiating an interphase just as well as you can a prophase or a metaphase. Whether the sensitivity is quantitatively the same, I haven't the slightest idea.

Another thing I can say with a fair amount of certainty, although I think I could find some loopholes in it (we never deal with certainties in biology, or very seldom) is that this radiation effect is due to a direct action on the chromosomes. I have an illustration of that in the final movie. (Movie) This experiment was done with a microbeam of protons, about 2 μ in diameter. Remember this chromosome group is about 20 by 30 μ in diameter. The cross hairs will show where just a few chromosomes were bombarded, right at this point. Just keep your eye on that area until the anaphase occurs. There it goes. Here is the point of bombardment. You see the stickiness is only there, not in any of the other chromosomes. We are getting a sticky bridge here, essentially the same picture we had awhile ago except instead of having practically all of the chromosomes involved we have only two or three, or maybe four. (End movie.)

If one wants to think up more complicated hypotheses he can always say that it is necessary to bombard the little bit of neighboring cytoplasm as well as the chromosome, and certainly that was done. But I think the simplest view is to say that it is necessary only to hit the chromosomes themselves, because we had a pretty good control in the other 21 or 22 chromosomes that were not hit. We have also done a lot of experiments in which we poured rather fantastic numbers of protons into the cytoplasm alone, keeping the beam completely away from any of the chromosomes, and we didn't get such an effect.[5] Incidentally, this effect was obtained with 60 individual protons. How many of them were effective in producing that stickiness I don't know. It may have been only a few.

There are other ways of producing aneuploidy instead of just producing stickiness. For instance, you can inactivate a kinetochore in a chromosome; when division occurs, that chromosome by chance may get into one cell or

the other. The cell that gets it has an overdose of that particular type of chromosome. The one that does not get it has an underdose.

Now I think here is a very pertinent question to ask: To what extent is this aneuploidy reflected in the cell's ability to perform? Here we see that at least it can perform reconstruction of the nucleus perfectly well. It comes in right on schedule. Even in a case where you inactivate a kinetochore and have essentially a floating chromosome, that chromosome will form a little nucleus of its own frequently and reconstruct at the same rate as the main nuclei, and those in turn will reconstruct at just about the same rate as normal nuclei. So aneuploidy evidently does not upset some functions at all. On the other hand, it certainly upsets the chromosome complement, and if this is in the germ line, I think almost certainly it is going to have pretty severe expression in the offspring. In somatic cells this is open to question, and I don't think we know very much about it. What is going to happen to this cell that we produced awhile ago where so many chromosomes were stuck together? Is that cell going to disintegrate later on? If it does disintegrate is it because of the aneuploidy? I certainly don't know. The point I want to make is that we don't want to jump at conclusions because we have something that looks pretty badly messed up microscopically.

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4. Radiation Effects on Mammalian Cells in Tissue Culture

C. M. Pomerat

I am sure all of you appreciate that the tissue culture world has become booming business. We had a sample of the exciting things going on in this area when we saw the marvelous work of Dr. Zirkle and his associates.

Stroud and Brues[1] pointed out in a review not long ago that radiation has been more thoroughly studied by means of tissue culture than any other toxic agent and that research work in this field has moved at practically a uniform, steady pace for 30 years. The accuracy of the results has increased with advance in techniques of dosimetry, and with the appreciation that the oxygen level at the site of radiation is a very important factor in cell sensitivity.

I am sure you are aware that with a variety of cell strains primarily of human origin and with many new techniques, there are now some fresh and important invitations to research. My purpose is to show you a few of the emerging techniques which lead us to believe that in time tissue culture studies are going to become more important in the field of radiobiology.

Our work is being supported under a contract with the U. S. Air Force. The irradiation of cultures prepared in Galveston is carried out at the Radiobiological Laboratory of the Balcones Research Center at Austin, Texas. Several manuscripts prepared by our group are currently being published.[2-5]

The method which we use for most of our radiation work involves a special kind of chamber, which was devised by Dr. George G. Rose. It is essentially a sandwich with a central circular opening 25 mm in diameter (Figure 1). It consists of two stainless steel plates compressing a pure gum rubber gasket. We can replenish the medium by the simple expedient of wiping the gasket with alcohol, inserting 25-gauge needles through the rubber and removing or replacing fluid or inoculating cells into the chamber. In this way we can grow monolayers of cells in a parallel-sided system with good optical qualities.

It becomes possible to follow changes in the form of cells immediately after irradiation with a variety of techniques using a perfusion chamber system. For example, in a still photomicrographic apparatus equipped with an incubator for recording such observations, one can observe a cell one day after receiving 2,000 r from a Co^{60} source, the same cell on the fifth day, and on the seventh day. In this way one can follow the development of lesions and of changes in the form of cells. We can also follow mitotic events with this technique. We are able to see giant cell formation resulting from 2,000 r of Co^{60} gamma rays, and we can observe the same cell at suitable intervals during the process of change. In a human epidermal cell, which was provided to us by Dr. Wilton Earle, we have regularly seen that doses of 2,000 to 4,000 r produce vacuolar bodies of this sort. I will talk more about

this lesion later when we show moving pictures of its genesis, which we suspect to be either a direct reflection of the radiation or which may represent the development of cytopathology due to the presence of masked viruses. It may be that with certain kinds of radiation techniques we can unmask viruses which are latent in many cell strains.

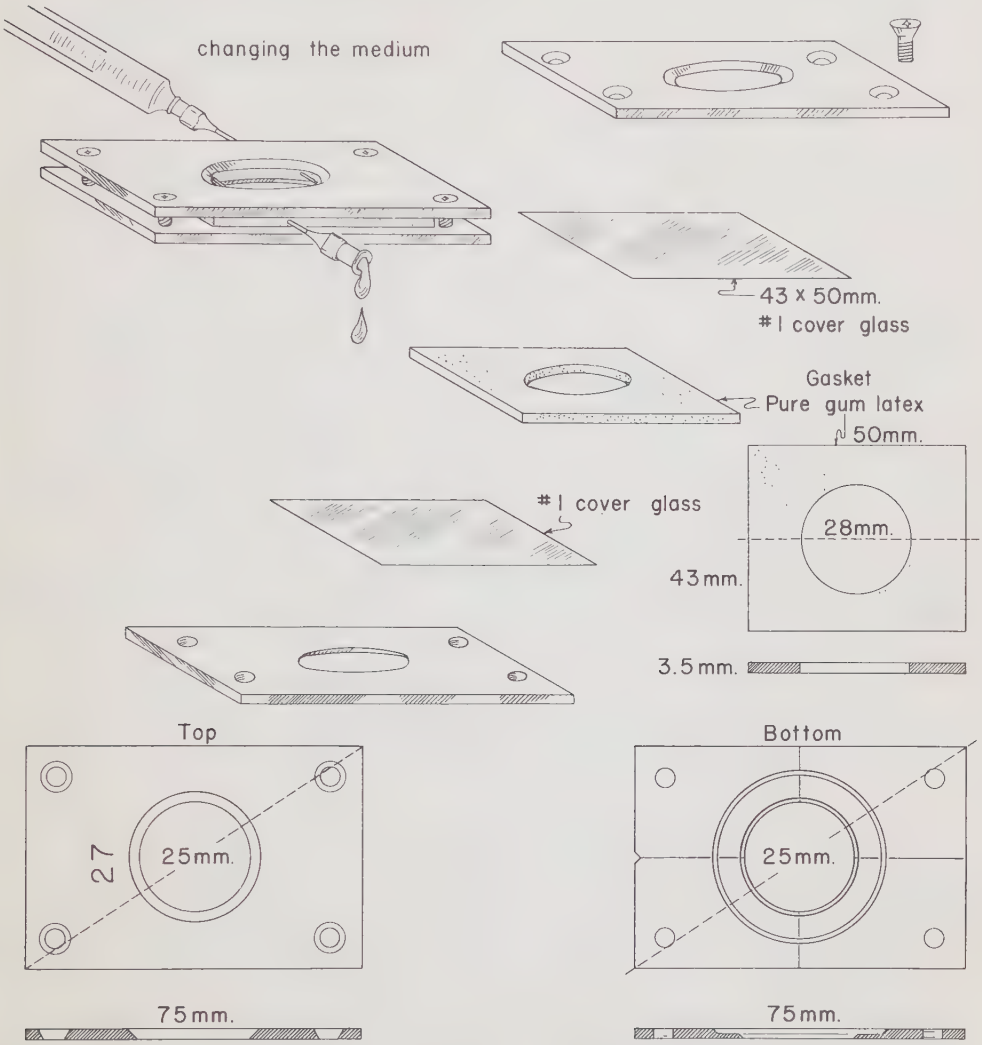


Figure 1. Diagram of the Rose chamber assembly.

Another method of studying these same things, but which follows more carefully the events taking place, is the time lapse cinematography technique that Dr. Zirkle described earlier. We placed Rose chambers on incubated microscope stages and photographed them at a very slow rate of speed, for example, one frame per minute, and followed the events taking place in the cultures for as long as seven days. I am not going to take the time now to tell much about what went on, but we observed a variety of anomalies and

followed exactly how they came about. In some of the discussion that took place earlier there were questions relative to increase in cell size, the matter of the swelling of the cells, and the process of giant cell formation. We followed particular cells after various radiation dosages and obtained the entire history of the cells. We have been able to follow teloreduplication, the phenomenon by which at the close of cell division one daughter cell flows back into the other, so that we get multiple nuclei and the development of giant cells, some with as many as 60 or 80 nuclei following various radiation dosages (Figure 2).

I am going to bypass a detailed discussion of this matter until later when you can see for yourselves the genesis of this anomaly in the moving picture sequences.

Cinematographic records have established that cells with single, double, and multiple nuclei can produce giants with varying numbers of nucleoli (Figure 2, i-r). Such elements which are generally considered to be "dead-end" cells occasionally were seen to divide a second time. Cultures of the skin strain which had received 4,000 r were followed with cine technique for 11 days. One of the elements, containing 6 to 8 nuclei, became rounded and after forming a multilobular mass spread out more than three times its original area. This giant contained approximately 60 micronuclei, the majority of which had single nucleoli (Figure 2, q and r). Rhythmic contractions of the cytoplasmic mass, which are frequently encountered after irradiation, persisted in this cell for approximately six hours. It is believed that irradiation per se did not create new anomalies but that it increased the frequency of abnormal cell types peculiar to cell strains.

As all of you are aware, the tissue culture method now makes possible the growth of cells in massive quantities. In a very common type of bottle used to grow cells in stock cultures, many human cell strains, both of malignant and nonmalignant origin, are available to us.

In essence then we have a system whereby we can raise gram quantities of human cells. These cells are available all over the world in connection with virological studies. The techniques of growing them have become very much simplified. We are very close to a completely defined synthetic medium for these cells. Certainly it is possible to maintain some, like the L strain of mouse fibroblast in a fully defined medium indefinitely.

Those of us working in the tissue culture field have become very much interested in the results obtained by Puck and his associates at Colorado[6-8]. I am sure that most of you are familiar with this work, but since Dr. Kaplan stated that we should also represent something of the work of others in our fields, I thought that it might be wise to discuss some of the work by Puck.

One of the phenomena that I want to concentrate upon has to do with the development of giant cells. Puck shows that as the dosage increases the average number of cells per colony is reduced and the proportion of giant cells increases. With 800 or 1,000 r, a population consisting practically entirely of single giant cells can be found.

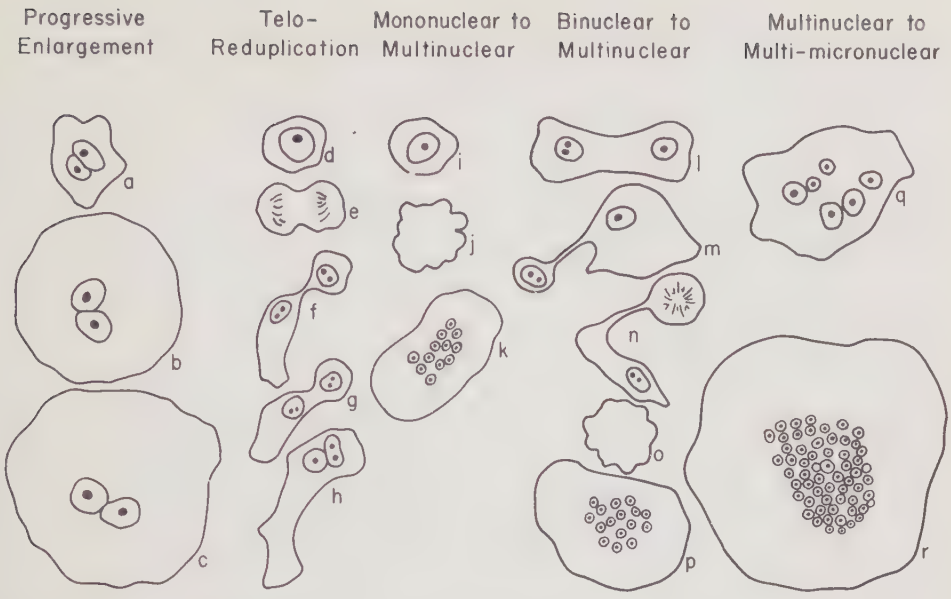


Figure 2. Types of giant cell formation as seen in cinematographic records of living elements.

The most common process consists of progressive cell enlargement. An individual cell (HeLa S3) photographed daily following 2,000 r of gamma irradiation typifying a gradual increase in area from the 1st day (a) through the 3rd (b) and 7th (c) days.

Teloreduplication is schematized from selected cine film frames recording the activity of a HeLa cell which received 2,000 r (d-h). A mononuclear cell (d) showing an apparently normal mitotic figure (e) reached the telophase (f) and, without accomplishing cytokinesis, one daughter flowed into the other (g) producing a binucleate cell (h).

Multinuclearity achieved by mononuclear and binuclear cells is illustrated by the diagrams labeled i-k and l-p of HeLa S3 strain elements which had received 2,000 r from a Co^{60} source. In these processes living cells (i) produced globular masses with a multilobed margin which were seen to be highly refractile with phase optics (j). This is presumed to represent a process involving a multipolar spindle resulting in a single cell with many nuclei (k). The example used to illustrate the formation of a multinuclear cell from a binuclear element (l) involved the separation of the original nuclei by a long slender strand (m). Divisional activity began in one nucleus well ahead of the other (n), but eventually a single mass was formed (o) leading to the establishment of a multinuclear element (p).

Giant cells with multiple nuclei are occasionally seen to undergo a second division. This is illustrated by the diagram of an element containing 6 nuclei (q) which became rounded and which showed "boiling" activity, ordinarily seen in cine records of the anaphase, to produce a cell with some 60 micronuclei (r).

It has become possible to develop clone strains, that is to say, strains of cells all of which are descendants from one individual. One is known as the HeLa S3 strain and it is being cultured now in many parts of the world. It was developed from the HeLa cell originally cultured by George Gey from a squamous cell carcinoma of the cervix.

By using the technique that Puck has devised in preparing a clone strain, cell strains have been obtained by Earle and associates using a modified technique involving conditioned medium and the cultivation of individual cells in capillary tubes. Puck was able to show that if you irradiate cells in a Petri dish and develop giant cells, they will serve to affect the medium in such a way that if you plant one individual nonirradiated cell you can start a colony.

This has very broad interest in terms of the whole question of cell nutrition and, more than this, the metabolic change which occurs with radiation. What is it that happens when you develop giant cells, what is it that comes out of these cells which apparently facilitates the development of clone strains, that is, populations from single individuals.

Using such techniques, Puck and his associates were able to get sensitive and accurate dose-response data for mammalian cells and they plotted survival of reproductive capacity in HeLa cells as a function of X-ray dose. In the plating technique it was observed that when 300 r were administered to a suspension of cells a very much smaller number of individual colonies grew after plating than among the unirradiated controls. In other words, it has now become possible to handle mammalian cells the way the bacteriologists plate out microorganisms.

BOND: Do those cells remain discrete like bacteria? Does each colony come from a single cell?

POMERAT: Yes, unless the colonies become confluent when they are located too close together.

BOND: How do you make your suspension? How are you sure they are individual cells?

POMERAT: It is trypsinized material. It is true that occasionally you might have two cells sticking together, but with very delicate trypsinization and proper shaking you can get pretty discrete colonies. If they are not discrete, you obtain dumbbell-shaped colonies in the same way as with bacteria. So you see the technique of plating cells is becoming a very exciting affair in the assessment of radiation injury to cells. It give us an objective way of getting at this problem. I am going to return, however, to the question of giant cells.

In another illustration a figure represented not colonies but individual giant cells. The HeLa cell may increase in volume as much as 15 times following radiation dosages of from 1,000 to 4,000 r. One of the most recent papers of Fisher and Puck deals with the way in which these giant cells affect

the environment. They showed that if an irradiated "feeder" layer was used, it would protect against an inositol deficiency in a rather well-defined nutrient medium. It is believed then that one of the ways that the feeder cell functions is by replacing the deficient constituents of the medium. Another way that it may function may be by neutralizing toxic materials. The irradiated giant cell apparently can absorb toxic substances, and Fisher and Puck present the following evidence for this: when an amount of toxic antiserum adequate to prevent the growth of unirradiated cells was used, the added presence of the irradiated cultures permitted the antiserum toxicity to be overcome and growth to resume.

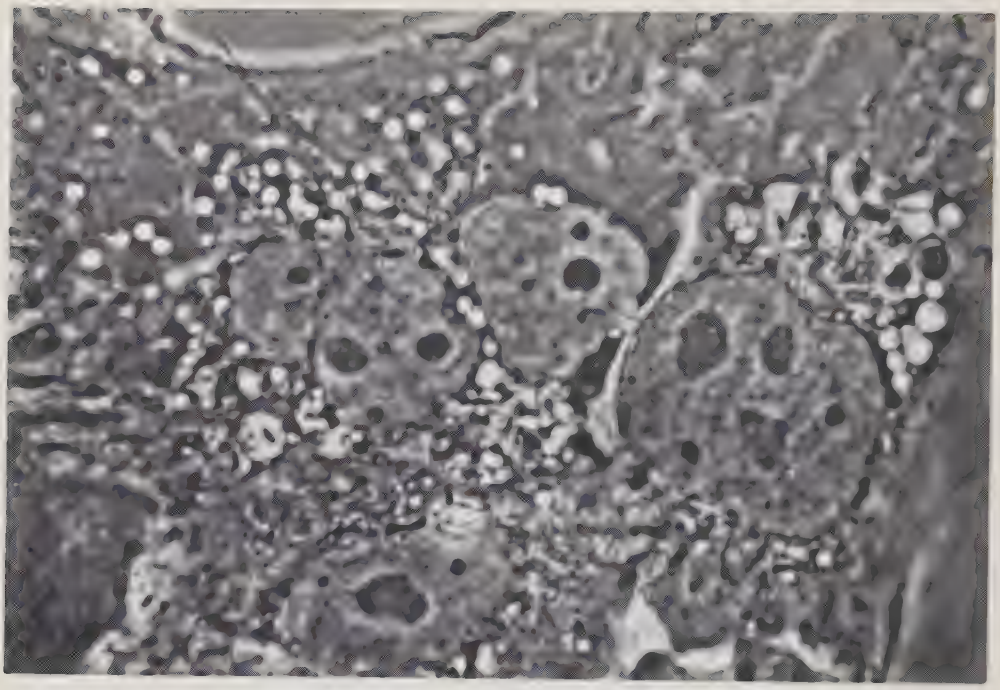
At the meetings of the Tissue Culture Association, held in April this year in Baltimore, Dr. Clement L. Markert of the University of Michigan described his studies of the biochemical differences between HeLa that was radiated and a standard HeLa cell strain. The data were obtained by recording with a Geiger counter the amount of radioactivity found in the various amino acid spots separated on a paper chromatogram. The amino acids were obtained by hydrolysis of the cells after growth for five days in a complex medium containing 10 microcuries of uniformly labeled glucose. The amount of radioactivity in alanine was used as a standard with which all the other substances were compared.

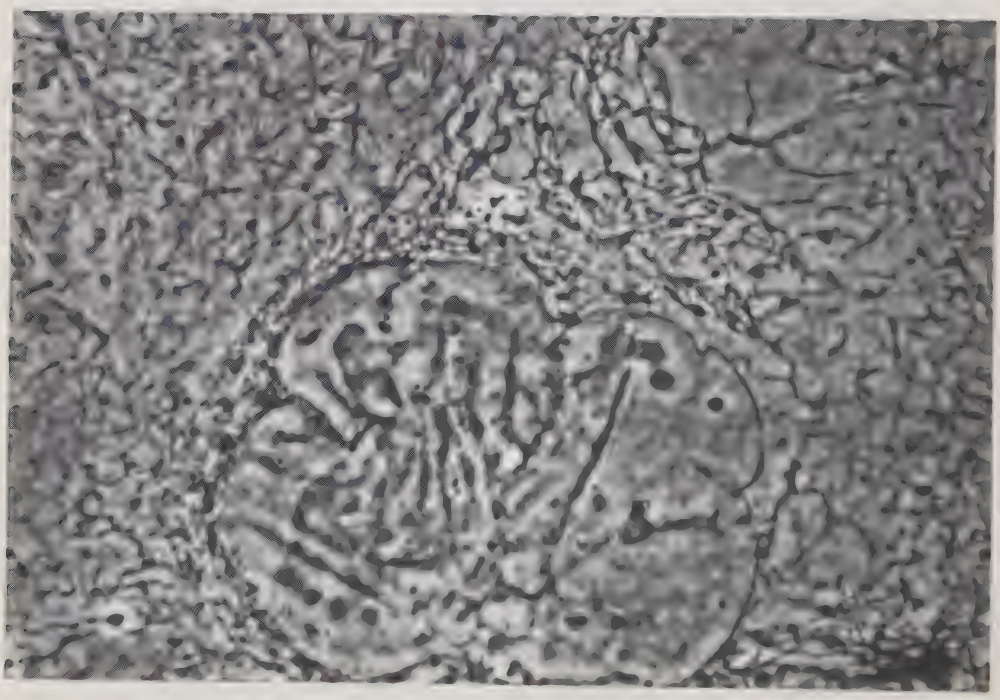
Thus, in the radiated HeLa there was 0.31 as much radioactivity in histidine as in alanine. Serine was also markedly affected. The relative amount of radioactivity does not correspond with the relative amount of the amino acid synthesized, since the number of carbon atoms per mole varies with different substances. Nevertheless the patterns of synthesis and metabolism are clearly shown in these data which also imply that the amino acid (and perhaps the protein) composition of the irradiated HeLa cells has changed markedly.

These, then, are some of the directions which tissue culture work is taking in trying to define metabolic differences between irradiated versus non-irradiated strains. Incidentally, this cell strain which is being used at Michigan maintains these metabolic differences in time.

We have irradiated a whole series of human cell strains in Rose chambers and fixed the cultures at the end of 12 days. In the upper row of three figures you see a cell which was derived from an adenocarcinoma of the lung and has been maintained for several years in vitro. (Slide) The top left picture shows the unirradiated control. The top middle picture shows the effect of 2,000 r, and the picture on the right, 4,000 r. Below we have corresponding pictures for the HeLa cell. We find that these two carcinomatous strains regularly develop a population of giant cells by the 12th day after 2,000 or 4,000 r (Figures 3 and 4).

Now we compare the genesis of giant cells after irradiation in strains originally derived from malignant cells with that in strains that began as so-called normal cells. (Slide) The liver strain of Chang is shown in the upper row. After 12 days you see the typical picture of the Chang liver strain with 2,000 and 4,000 r. Extraordinary anomalies of nuclei are developed. This is also true for the lower part of the picture which shows Eagle's gingival strain. That, incidentally, originated as a carcinomatous strain.





Figures 3 and 4. Comparison of living HeLa cells of the S3 clonal line (Fig. 3) with similar elements 25 days following 2, 000 r of gamma irradiation from a Co^{60} source (Fig. 4). Both illustrations at the magnification given by the scale in Figure 3.

In summary then, 11 cell strains used in these experiments (derived from 9 different cell lines) whether they came from malignant or nonmalignant cells, all produced giant cells within 12 days following 2,000 or 4,000 r.

In contrast to this we grew a series of primary explants, that is, cells which were not accustomed to living in vitro. We were never able to produce giant cells with primary explants. This is true for a long list of tissues. I won't take time to list them.

As you know, with tissue culture methods it is relatively easy to spread out the chromosomes, using a hypotonic solution applied 20 minutes before fixation of the culture. In this way we can count the chromosomes very nicely.

Another illustration was given in a recent paper by Dr. Alice Moore.[9] In Levan's laboratory human chromosomal drawings were made to show what the situation is in embryonic lung fibroblasts and human chromosomes from the Chang liver strain. When cells are cultivated in vitro for some time, they become polyploid or at least aneuploidy develops. In HeLa cells, for example, in 200 counts we never encountered the 2-N number of chromosomes; instead, there is a hypotetraploid distribution. We observed also a fairly suggestive correlation between chromosome numbers and the DNA values for similar tissue.

If we start with primary explants we deal principally with cells in the 2-N condition, and when we irradiate these we do not get giant cell formation. But when we irradiate the transformed, usually polyploid strains we do get giant cells in all of the cases that we have tried.

Another case showed the change in chromosome number in the series of subcultures that were made from joint tissue. Up to the fifth subculture the majority of the chromosome counts ranged from 40 to 50, but between the fifth and sixth cultures some process became deranged so that the count shifted to something above the tetraploid number by the ninth culture. On the ninth subculture the average number of chromosomes is around 130.

Now in order to check this problem, and after discussion with Dr. Wendell Stanley, we decided to study the strains of amnion cells which Stanley's group had established. We found, as we expected, that the amnion strain would develop giant cells. But when we tried amnion cells in primary explants we were surprised to find that they became giants too. We think perhaps in the case of the amnion cell we may be dealing with a normal condition of polyploidy, and we are pursuing this question. There may be certain other areas--perhaps the dome cells in bladder epithelium are in the tetraploid state also. In such situations we may get giant cells after irradiation of primary explants.

In summary then, giant cells regularly develop in irradiated cell strain cultures which have been proven to have an aneuploid or rather a heteroploid condition. We have not encountered giant cell formation from cells of primary explants except in the case of the amnion cell. Unfortunately, we do not yet know the chromosomal condition of normal amnion cells but this is being studied.

I want to discuss one more kind of experiment before closing, and this is the attempt to do dosimetry with tissue cultures using a test tube technique.

As you know, in virology the standard practice is to seed the test tube with 100, 000 cells suspended in one milliliter of nutrient medium. These are put in racks and are allowed to stand for a day, and then the material containing virus particles is added, usually to groups of six tubes at a time. We have done this same sort of thing with radiation techniques. Then we irradiated groups of six at a time, and we studied them with the same techniques as the virologists are using, notably the change in pH and the appearance of cytopathology.

We also observed changes in pH as a function of time. The pH was plotted as the difference between the pH value read on the first day and the pH values on subsequent days. The change is a function of dose of irradiation from a Co^{60} source up to 80, 000 r. This can be plotted as the pH value directly or as the difference, which irons out some of the initial irregularities. We observed that our control pH change was very rapid with no irradiation, but with as little as 120 r there was a detectable difference, and the change across the range from 1, 000 to 10, 000 r was relatively constant. At 20, 000 r the injury is so great that there is almost no pH change, while at 50, 000 r all cells had probably been destroyed.

In summary, we think this may turn out to be a very useful method not only for simple dose assay, but also in the quest for protective agents. In contrast to the use of whole animals then this is simple, relatively inexpensive, and very rapid.

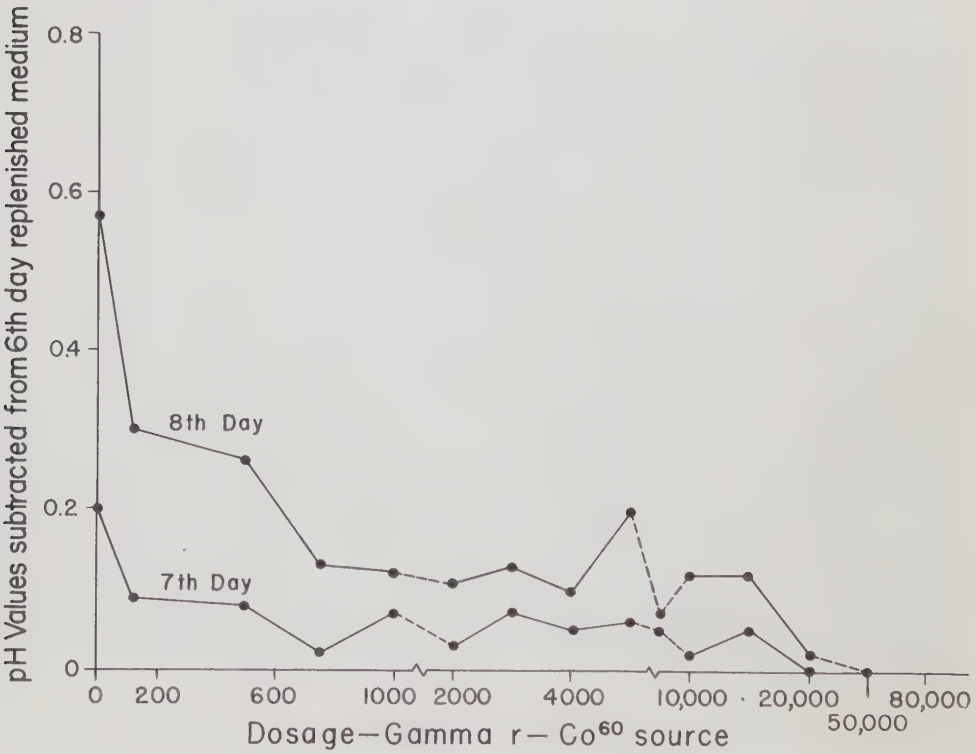


Figure 5. Results obtained with test tube method for the study of radiation injury. Each value represents the average reading of six similar tubes each originally containing 100, 000 HeLa cells of the S3 strain per milliliter.

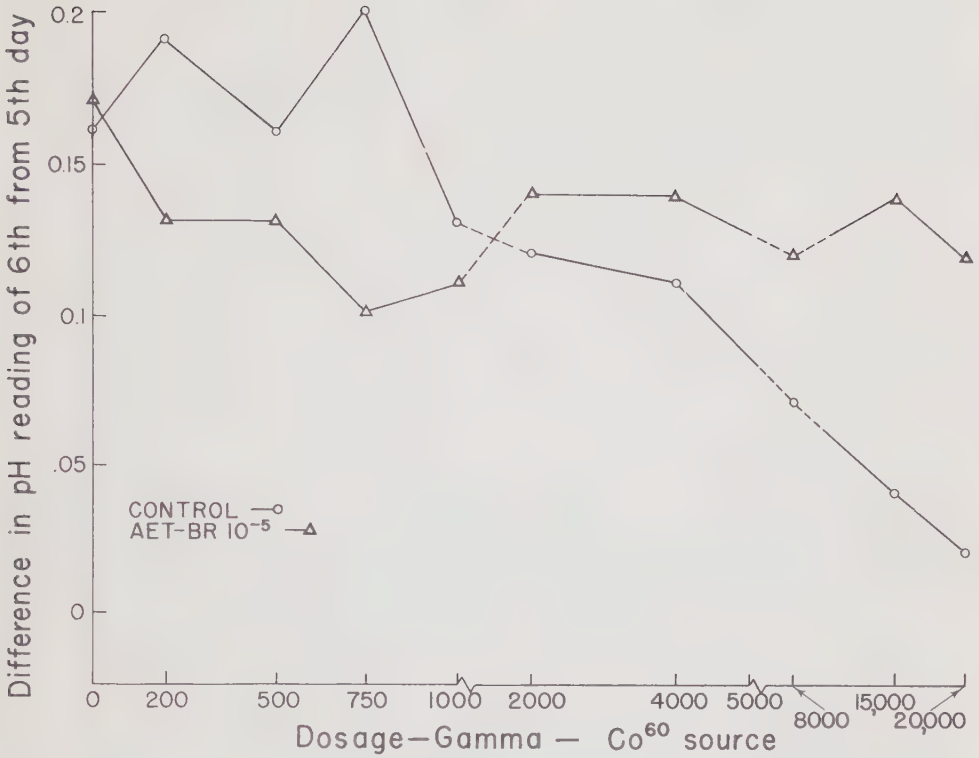


Figure 6. An example of results obtained with the same method as described in Figure 5 but illustrating protection against injury between the 5th and 6th days post irradiation by S, β-aminoethylisothiuronium · Br · HBr (AET) at 10^{-5} which had been in the medium 2 days prior to the exposure of cells in a Co^{60} facility.

NICKSON: Dr. Pomerat, how much is known about the oxygen tension under conditions of radiation in tissue culture, and what can be done to modify oxygen under controlled conditions?

POMERAT: Cultures in Rose chambers which give monolayers of cells are ideal for oxygen experiments. What we found early in the game was that when we irradiated with the fluid nutrient in the chamber, we got a value, for the total destruction of the cells in 24 hours, of approximately 120 r. But when we removed the fluid and irradiated, about the same effect was achieved with 80, 000 r. Lately we have been studying the difference in injury when we irradiate the chamber when the fluid is replaced with air versus 96 percent nitrogen and 4 percent CO_2. We find a very real difference in the giant cell production in the presence of air versus this low oxygen mixture. This result was not appreciably greater after substituting pure O_2 for air. We have begun studies of the relative susceptibility of cells of malignant versus non-malignant origin to changes in O_2 level during irradiation.

J. R. ANDREWS: May I ask if it is possible to induce snychronous division in these clonal strains?

POMERAT: So far as I know, no one has been able to get synchronous mitosis in tissue culture, even though the tricks of repeated chilling and warming have been tried. This has been a very important goal that no one has been able to reach.

PATT: What is the order of difference between air and 96 percent nitrogen?

POMERAT: I think with 750 r, if my memory serves me, in air we got 75 percent of the cells becoming giants. With 96 percent nitrogen and 4 percent CO_2, 25 percent of the cells became giants.

KAPLAN: One might ask whether these are still human cells after they have been transformed.

POMERAT: A recent article by Alice Moore in the Transactions of the New York Academy of Sciences,[9] in which she makes a provisional statement on the Chang liver strain implanted in individuals with terminal malignancies versus subjects in penitentiaries, leaves her begging the question. She is not sure. Certainly in the patients with malignancies solid masses of tissue grow, although they have not been followed to check for metastases, whereas in the penitentiary volunteers, implants have not grown into nodular masses.

It is true, too, that in animal experimentation cells of malignant origin form a larger number of solid tumors than the so-called normal cells when they start. If you use the criterion of homo-transplantation they behave like human cells.

ZIRKLE: Does this production of giant cells in the animal preparations suggest that there might be a lot of aneuploidy in those tumors that seem to produce giant cells and have a tendency to proliferate?

POMERAT: While I know there has been a lot of controversy among radiotherapists concerning the work of Graham,[10] I have found this a very challenging notion. We are dealing with tumors of very mixed cell populations. We believe the markedly aneuploid cells are the ones which produce giants. All of these factors must be weighed in the equation and I think we are getting tools whereby this can be done. For instance, in the last issue of Cancer Research, Shields Warren and Olive Gates,[11] irradiating human tumors transplanted to the hamster cheek pouch, find great differences in radiation effect in different parts of the tumor. One wonders about the proportion of cells which go into the giant phase. Since they have an altered metabolism which stimulates proliferation, they may actually excite their neighbors to greater mitotic activity. All of these are pertinent questions for the radiotherapist.

ZIRKLE: Have you ever seen any mitosis in giant cells?

POMERAT: In giant cells? We work primarily with 2, 000 and 4, 000 r and we do see highly abnormal mitosis in such cells. Puck has claimed that 4, 000 r stops all mitotic activity as measured by colony production on plates, but we think cells so treated do occasionally divide. I have evidence that 4, 000 r does not prevent nuclear division, but I have never yet seen the cells survive beyond the second abnormal division. Usually they explode as we will show you later in the films. While they can go through a second division, they apparently can't survive.

ZIRKLE: After they go into the giant cell stage and become multinucleates, you don't see mitosis in those cells at all.

POMERAT: Yes.

ZIRKLE: You do see them?

POMERAT: I can show you this in the moving pictures in the case of Earle's skin strain. He prefers to have us call it the NCTC 2414-12-4 strain. The film shows a cell with at least six nuclei of various sizes. Unfortunately, in this material we cannot see the chromosomes or spindles because the cells become globular and highly refractile. But in the anaphase we see them settle down as single giant cells with as many as 60 nuclei.

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II. FACTORS IN THE HOST AND TUMOR BED

1. Role of Nutrition, Vascular Bed, and Stroma

Henry S. Kaplan

So far we have been talking about the cellular aspects of radiosensitivity and it is quite obvious that we have only scratched the surface. We probably could have devoted the entire conference to this aspect. It is conceivable that some of you may feel that there would be virtue in expanding this to make a future conference and we would be happy to hear from you one way or the other about this.

It is necessary to go on, however. It seems logical next to consider these factors within the host, and specifically in the tumor bed, that might condition the radiation response of the tumor. I am going to touch very briefly on some factors having to do with nutrition and the vascular bed. I don't know anything about the influence of stromal cells, so I won't say anything about that topic.

There is really very little direct evidence about the role of the vascular bed in radiation response. A few years ago, with Drs. Ruth Merwin and Glenn Algire at Bethesda, I did some work which we published in 1950,[1] and this is simply a very brief review of some of the major features observed in that study. I think this is extremely incomplete and that much more work is needed along this line. The work is so painstaking and slow and difficult, however, that it is hard to make much progress with it. To the best of my knowledge, Algire's group is the only one that is really technically equipped to pursue this type of study at the present time. I should make it clear that most of the actual observations described in our paper are primarily the painstaking work of Dr. Ruth Merwin.[2]

We used Algire's adaptation of the transparent chamber method, which consists of making a skin flap in a mouse, removing one layer of the double thickness fold of skin, so that there is a denuded area on one side which one covers with a clear mica window which can be fastened in place by some rather ingenious techniques. Beneath the window one can implant a tiny fragment of tumor or of normal tissue. You can watch this implant under the microscope with the mouse held in a special metallic holder through which the skin flap protrudes so that it is under the microscope and the mouse is lying off to one side. You can then pick up this mouse again at any subsequent time and place him back under the microscope and reproduce your coordinates to observe the same areas under the window each time. It is possible to make time lapse movies or intermittent observations on blood vessel development and the sequence of changes in the blood vessels of a tumor more or less continuously for quite a number of days. Some of these observations went along for as long as two months.

We used experimental mammary carcinomas of mice. These were implanted under the mica windows. We either irradiated the bed, that is, the window area before putting the tumor in, or the tumor after it had started growing in the area under the window, usually with single doses in the range of 2,000 to 3,000 r. The changes, unfortunately, must be described in descriptive rather than quantitative terms.

Through a typical window there is a tumor growing, and one can see the blood vessels fanning throughout the tumor. One point worth calling attention to is the rather uniform ramifications of the dividing capillaries throughout this tumor, with perhaps some decrease in number of vessels near the center. One might very well wonder whether this was not a relatively anoxic zone in the tumor at the time of irradiation. Around the periphery, however, there is good homogeneous vascularization.

ZIRKLE: How big is the tumor?

KAPLAN: This is on the order of 1 or 2 μ. Some of them got a little bigger than that by the time they were irradiated.

Three days after 2,000 r there was a striking change in the caliber of the vessels. We did not interpret this as a primary effect on the vessel. We interpreted this as a decrease in the requirement for nutrition by the radiation-damaged tumor cells. There is other evidence which Algire and Merwin have, unrelated to this study but obtained in similar chambers, which would indicate that when the tumor stops growing the blood vessels tend to decrease in caliber. So the change in size is probably a reflection of a change in functional need of the tumor at this point in time.

However, the vascularity in one or another area tended to increase a little or at least remained relatively full. This area was the one that soon showed renewed tumor growth, while the rest of the tumor was undergoing necrosis. So the persistence of vascularity here at this very early phase may indicate that these cells have been less heavily damaged.

PATT: That does not correspond to the anoxic area.

KAPLAN: No, it does not correspond in this instance. Ordinarily the other kind of thing happened.

In a tumor nine days after irradiation all that one sees are areas of tumor that were originally translucent and have now become opaque. They look dead. You see the fragmentation of the blood supply at nine days. In the same tumor at 16 days of irradiation areas that were apparently dead have begun to grow again. We noticed particularly the dilatation of these capillaries in the regrowing zone of tumor. When a normal, nonirradiated tumor grows you see new capillaries budding in between the existing capillaries, so that as the tumor expands its cells are continually provided with a fresh blood supply. In this irradiated tumor, new capillaries failed to develop. Instead, the existing capillaries dilated progressively in order to take care of the need. In an area that had started to regrow, we noticed that

as it expanded these vessels just got bigger and bigger, but the new bridging vessels that one might expect to develop simply refused to appear. Radiation injury seems to affect the capacity of the capillary endothelium to bud new capillaries. Ultimately stasis appeared in this focus of regrowing tumor. Apparently a delayed circulatory block had been induced by the irradiation, which resulted in a secondary wave of necrosis from circulatory impairment.

If host vessels from an unirradiated portion of the bed now came into this necrotic zone, capillary growth was excited by a granulomatous reaction in response to the necrosis. When the new vessels reached the tumor, areas that had apparently been dead and opaque for several days suddenly became translucent again, and the tumor cells started to grow at a perfectly normal rate. Thus in an irradiated tumor you have also the semantic question: when is a tumor cell dead? I think we are all aware in clinical practice also that areas which have been irradiated, apparently effectively, and are later cut across surgically or traumatized in such a way that a new blood supply comes into them, may show renewed activity when they have apparently been dead for many years.

In one example of irradiated tumor growing again we noticed that as it expanded (the expansion was very obvious) how this very narrow capillary became dilated. Stagnation was beginning to set in.

In an experiment in which the normal bed was irradiated first and then a tiny piece of tumor was put into the chamber later, the abnormal-looking vessels were simply damaged by the process of insertion of the tumor fragment. The rest of the vessels were those of the normal host, but they had been irradiated. We observed that as the tumor grew the vessels coming into it no longer exhibited innumerable fine arborizations. They remained very few and became quite enormous. This indicated again that the irradiated vessels of the host bed were unable to supply the tumor because they could not bud new capillaries.

The other environmental aspect that I want to touch on is the question of nutrition. Not very much has been done with this. We are all aware, clinically, that patients who are cachetic and debilitated tend not to do well with radiotherapy.

A few years ago Elson and Lamerton and their associates[3, 4] at the Chester Beatty Institute did some work with the Walker carcinoma, grown as a transplant in rats and treated locally with X-rays. Some were on a 20 percent protein diet and others were on a 5 percent protein deficient diet. You can see the schedule of fractionation. I don't know why this scheme of fractionation was pursued. The solid lines are the animals kept on the 20 percent protein diet and the dotted lines are those on the 5 percent protein deficient diet. Initially the irradiation response is about the same, but what finally happens is quite different. The tumors in the 20 percent protein-fed animals seem ultimately to disappear, whereas the tumors in the protein deficient group, after initial suppression of their growth, start growing actively again and kill the animal. The animals on the low protein diet seem unable to keep permanently under control tumors that have been heavily

irradiation damaged. In contrast, under good nutritional conditions the other group of animals were able to dispose of any vestiges of irradiated tumor.

This has always struck me as an extremely interesting observation. I was, therefore, a little perturbed to learn from some British colleagues visiting in this country recently that this work had not yet been confirmed. I don't really know whether that is true or not. But I think if it has not been confirmed it is quite important that it be restudied.

Another illustration shows some typical histological findings in such an experiment (this is at 4, 000 r). The animals of the high protein group tend to form a very well differentiated fibrous capsule around the tumor as it is regressing. This is a very dense capsule whereas in a similarly damaged tumor in an animal on the low protein diet the fibrous capsule is very thin and pervious. It was their thought that perhaps the lack of stromal defense reaction was what was missing.

I think it is important that this kind of work be opened up again. We have taken a somewhat different approach, feeling that the study of nutrition in a live animal, or in a live human for that matter, is a very difficult and complicated business. We thought that we might at least get some preliminary clues from a study of the influence on radiosensitivity of altering nutrition in mammalian cells grown in tissue culture, using as completely defined media as are available at the moment. This work is still in its incipient stages.

In summary, I think the role of nutrition is still very imperfectly understood and that it may turn out to be a very large factor in cellular radiosensitivity.

I will now call for further comments from Dr. Curtis and Dr. Vermund unless some of you have questions.

BOND: Did the vascular bed ever regenerate during the entire period you looked at it?

KAPLAN: No. Ultimately the windows break down, after a couple of months or so. For as long as we were able to follow them, however, the only time the tumor regrew later was when it got a new blood supply from an unirradiated site.

One other observation that fits is that in some instances we put embryo tissue alongside of tumor tissue. Of course, the embryo tissue can form its own blood vessels. We put both of them on a pre-irradiated bed. The pre-irradiated bed of the host did not supply vessels to either the embryo or the tumor, but the embryo made its own vessels, connected them up with those of the host and grew nicely. Pretty soon it made contact with the tumor and supplied the tumor with vessels, at which time the tumor finally blossomed forth and grew at the standard rate.

CURTIS: This is a subject I have been interested in for a long time but

from a different point of view; namely, premature aging induced by radiation. A number of lines of evidence could be cited to indicate that severe vascular change takes place as a result of radiation and these vascular changes remain as fixed changes so once a tissue, especially skin, has received a severe dose of radiation, the vascular bed will be permanently altered. Some of the evidence which Dr. Kaplan has just been presenting illustrates this very nicely.

However, almost all these observations are descriptive observations taken from histological material. Unfortunately, there is almost nothing in the way of physiological data to back up these observations so it is impossible to say at the present time whether the altered vascular bed is a result of changes in the tissue or whether the tissue has been forced to change to accommodate itself to an altered vascular bed.

KAPLAN: Dr. Vermund has made some studies along these lines, and will carry on from here for a little while.

VERMUND: The experiments that I am going to report on were carried out under the direction of Dr. Wilhelm Stenstrom[5,6] at the University of Minnesota. He suggested that we should reinvestigate the so-called tumor bed effect. Many papers have appeared on this subject, the first one in 1914 by the Austrian investigator, Frankl; later in this country by Murphy, Sugiura and others; in Denmark by Krebs; and in England by Russ and Scott. They all arrived at the same conclusion, that local irradiation to a normal tissue followed by implantation of fresh, nonirradiated tumor resulted in retardation or complete inhibition of growth of the implanted tumor.

The early investigators worked with animals which were not inbred to the extent that we have them available today. Furthermore, the radiation dosage was not well established. So we thought that it was worthwhile repeating these experiments to see if we could confirm them. We were in the fortunate situation of being able to work with Dr. John Bittner, who has a large colony of inbred mice of the C_3H strain or, as he prefers to call them, the Z strain.

We took the spontaneous mammary cancers that developed in mice of this strain and implanted them into back-cross ZBC mice. These animals had been given irradiation locally to one leg. We also ran controls of course, and compared the rate of tumor growth as a measure of the effect of this so-called pretransplantation radiation.

In one series of animals one leg had been irradiated with a dose of 1,500 r, and then 48 hours later fresh, nonirradiated mammary carcinoma were injected into both legs. In every single animal we found that there was marked retardation of the growth of the tumor transplants in the irradiated leg as compared with the shielded opposite side.

In a control series where we got equal growth in both legs, these animals had no irradiation but were of the same strain and were injected simultaneously with the same tumor. We plotted the growth curves after

having repeated this particular experiment ten times. In all experiments we got similar results. The curve indicated the rate of growth of the tumor implanted into the irradiated leg. If we implanted only the irradiated side and studied the survival of these animals, it became apparent that pretransplantation irradiation could not completely prevent the growth of these tumors. In a survival curve following pretransplantation irradiation, the controls started to die off in the sixth week, and as time went on more and more died. With some dose levels of pretransplantation irradiation there were prolonged survival; we obtained the best effect with 3,000 r, but even that dose would not completely prevent ultimate tumor growth, and with the higher dose of 4,500 r we got less effect. Actually some of these animals might have died from the radiation effect after 4,500 r. Our control animals had a pretty constant average survival, calculated from the time of the transplantation. The animals which had 1,000 r, 1,500 r and up to 3,000 r had somewhat increased survival, but the difference was not very great when the one leg was inoculated.

KALLMAN: Is that given as a single dose?

VERMUND: Yes, it is given in one single treatment.

Next we wanted to compare the effect of radiation given prior to the inoculation with irradiation given after the tumor cells had been inoculated. These mammary carcinomas are very resistant to radiation. Goldfeder, Ting, and many others have observed this and we confirmed it. We found that even with the very high dose of 6,000 r although we got initial regression of the growth, in every single instance there was a recurrence at the site of inoculation. We didn't try to give any higher dose because sometimes it was not compatible with the survival of the animals.

If we study the average survival of these mice that have received post-transplantation radiation of a tumor which has become established, that is, more than three weeks after the transplantation, we find that the average survival of these animals is in the same range as we found for the animals that had pretransplantation irradiation.

In direct comparison, we found that the average survival figures were pretty close in the groups receiving 1,500 and 3,000 r. There is a little difference with 4,500 r, and a somewhat greater difference with 6,000 r. However, if we gave irradiation within two weeks after the tumors had been inoculated, we observed that in a great number of mice the tumors could be completely prevented from growing and the average survival of the animals was markedly prolonged.

With a dose of 3,000 r, using a tumor which had been transplanted over 20 generations and was very malignant and rapidly growing, we got an average survival in the controls of 5.8 weeks. With pretransplantation irradiation, the increase in average survival was not very marked (7.7 weeks). If we gave radiation soon after transplantation the increase in survival was greater (16.4 weeks). However, if we waited 21 days after transplantation we actually didn't get any increase in the survivals as compared with the controls.

In a similar experiment following a dose of 4,500 r using a tumor which had been transplanted only twice, we found that in the controls the average survival was only about nine weeks. With pretransplantation irradiation it was from 10 to 14 weeks. But if we gave irradiation right after transplantation the average survival was 44 weeks, and many animals are still living without palpable tumors. If we delayed irradiation until 21 days after tumor inoculation, the average survival again dropped to about the same range as with pretransplantation irradiation.

In an experiment that we just completed, we gave 4, 500 r and used the same tumor in the controls in the animals that had pretransplantation irradiation and in those that had posttransplantation irradiation. There was a very marked difference in the survival and a number of the animals that had had posttransplantation irradiation a short time after the inoculation never developed tumors during the period of observation. Some of them died without tumors after having lived an almost normal life span.

In conclusion, posttransplantation irradiation given a short time after the inoculation of these tumors can prevent or delay the growth of tumor. We have observed in a few animals, a total number of 10, that tumors may develop as long as one year after immediate posttransplantation irradiation.

The interpretation of these experiments is a different story. We like the working hypothesis that a tumor that has become attenuated from the direct effects of irradiation can be destroyed by mechanisms in the organism. We don't know what these are. They may be humoral or cellular defense mechanisms which will not exert an appreciable influence when nonirradiated tissue is inoculated. However, they may become apparent and attack attenuated cells which have been irradiated a short time after the inoculation.

KLIGERMAN: The tumors at 21 days were considerably larger than the tumors at 2 days. What influence do you think that has?

VERMUND: We believe that the tumor size is of great importance. Dr. Nice, working in our department, has shown this to be true with transplanted lymphosarcomas. It takes a much higher dose to eradicate a large lymphosarcoma than a small one. With these mammary tumors we have a definitely palpable nodule after three weeks. After two weeks we can hardly feel it.

BOND: Do these tumors ever metastasize?

VERMUND: These tumors do metastasize.

BOND: Is there any difference in the rate of metastasis under the various conditions that you use?

VERMUND: We have not studied this problem, but Dr. Martinez, who is working with Dr. Bittner, has done some studies on it. He has implanted the tails of mice, and by chopping off the tail at different times after the inoculation he has found that metastases do occur relatively early, perhaps two to three weeks after the inoculation.

BOND: Does the irradiation have any effect on the frequency of metastasis?

VERMUND: We have not studied that. There are some studies from Denmark. It has been claimed that if you irradiate the transplanted tumor with a dose that is insufficient to eradicate the tumor completely, the incidence of pulmonary metastases is increased.

KAPLAN: I think you are quoting some of our old work[7,8]. This was confirmed by Kaae[9] in Denmark.

GARCIA: I noticed you mentioned that with the larger dose there was a drop in the length of survival when you gave it at one sitting.

VERMUND: We have made a lead cone that we can put right on the X-ray machine. It has holes in the walls toward the bottom, through which we pull the legs, allowing us to irradiate the tumors locally with less total body scatter. We don't believe that we can completely eliminate stray radiation to the body, and we feel pretty certain that some of the increased mortality that we get with the higher dose levels is due to the total body effect.

GARCIA: I might mention a clinical observation in connection with this. In carcinoma of the cervix our policy for awhile was to give rather moderate dosage to the periphery of the pelvis, about 3,000 r in about 30 days; subsequently, with the idea that by increasing doses to the pelvis you can improve the result, we increased the average dose to about 4,000 r. There are something like 500 cases in each group. We noticed that after the fifth year the incidence of recurrences was much greater in the patients who had had the higher dosage to the periphery of the pelvis. That is, of patients who had 3,000 r plus radium, the incidence of recurrence between the fifth and tenth year was 6 percent whereas in the patients who had a higher dosage it went up to 18 percent, which in groups of this size was significant.

QUESTION: What were your survival rates?

GARCIA: Do you mean at the end of the fifth year?

QUESTION: Yes.

GARCIA: At the end of the fifth year they were very much alike. In the tenth year, of course, the survival rate in the higher dose group was smaller.

KAPLAN: Was there any difference in distribution of stages?

GARCIA: The patients who had the lower dosage had the worse stage distribution.

POMERAT: I think this is an interesting point on which to raise questions as to methodology as between in vitro and in vivo experiments. In these fine papers we have seen how one can approach certain fundamental questions with the organism as a whole. In Dr. Kaplan's and Dr. Algire's

work we see that unquestionably we are dealing with a problem of availability of nutrients. In the tissue culture, presumably there are enough nutrients under the methods we use, so that we are supplying essentially what the vascular bed supplies. With respect to the paper by Dr. Vermund, I think this is the kind of thing which might be subjected to analysis with in vitro methods. We might be able to work out minimal radiation injury doses for a culture of a given tumor cell alone, and then place it in conjoint culture with a variety of cell strains. In the presence of heteroploid elements, we might find an enhancement of the growth rate at certain radiation dosages in terms of the Puck feeder layer system. In contrast, no such stimulus might result from contact with normal mesenchymatous or stromal elements. In other words, I visualize that your whole scheme can be set up experimentally in vitro and perhaps directed at very specific cell systems. The conclusions you seek, which are of utmost importance to the radiotherapist, may be established by such controlled experiments. It may be possible to alter tumor cell vulnerability to radiation by some as yet unknown defensive mechanism.

J. R. ANDREWS: That isn't what those data show, is it? Is that the conclusion we draw from this talk?

VERMUND: That was the tentative conclusion, I would say.

J. R. ANDREWS: But the greatest effect was when the tumor was radiated immediately after or shortly after implantation, indicating a greater effect on the tumor per se.

VERMUND: Yes, there was a greater effect.

J. R. ANDREWS: We know from some of our own experiments in which an extremity of the growing rat has been radiated that this has quite a profound effect on the whole growth of the animal. You have produced a sick tumor bed. I don't define what a sick tumor bed is here, for there are many things which are not evaluated and which may be of importance in local as well as in general immunological responses.

VERMUND: That is true. It is a very crude experiment. It needs a lot more work and we have more experiments coming. But we like to emphasize the fact that if you take the number of ions produced per tumor cell, you should actually expect the biological effect to be the same regardless of the time after inoculation at which irradiation was given. The fact that this does not hold true suggests that there is some other mechanism which adds to the destruction of the tumor and this mechanism we think might originate in the host. This is a working hypothesis. We have no proof of it.

KAPLAN: Of course, during the first two days after the tumor cells are implanted into a new host the cells are devoid of circulation and are in an anoxic state. Despite the anoxia they are extremely vulnerable. They don't have a nutritional supply. They are in a foreign host. They are in a very unstable dynamic state, and the effect that you have described has at least a partial counterpart in the field of cancer chemotherapy. It is well known that if you want to score a chemotherapeutic triumph the best way to

do it is to treat leukemia at a time when the definition is a little obscure. If you give an animal leukemic cells at time zero and then you start to inject the chemotherapeutic agent at one hour, the animal does not have leukemia at one hour. He has simply been inoculated with leukemic cells. Strictly speaking, this is not prophylaxis, since he always dies of his leukemia; he just takes longer to do it, and the drug effect represents neither prophylaxis nor therapy.

There is, I think, a very definite parallel here. It has to do with the unstable host-tumor relationship that is present very shortly after the implantation of the tumor. This, of course, raises one other problem. This sort of work is terribly important, but I think that we should all be aware of the pitfalls of working with transplantable tumors. I think there is much to be learned from them, but basically the transplant and its host are foreign bodies to one another, even if they come from the same strain.

I think there is need for much more work with tumors, either induced or spontaneous, arising in the original host. Recent evidence which strongly supports this view is the work of Scholler, Philips et al[10], at the Sloan-Kettering Institute. They showed that transplantable mammary tumors of a C_3H hybrid could be very effectively treated with chemotherapeutic agents as transplantable tumors, but when they treated the same histologic variety of tumor in the original host in which it appeared as a spontaneous tumor, the same chemotherapeutic agents were entirely without effect. They didn't have the slightest influence on the growth of the tumor. We have there a very important reminder that there is something rather artificial about all transplantable systems. I think we should use them for what they can tell us, but one has to be very aware of the pitfalls.

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2. Oxygen Effect and Modification by Oxygen Binding Agents; Theoretical Basis of Gray's Proposal for Use of High O_2 in Therapy

Harvey M. Patt

The relationship between oxygen and radiation responsiveness has been the subject of considerable investigation and much speculation. I should like to discuss with you the oxygen effect and some of its theoretical and practical implications.

First we might define the oxygen effect. In essence the oxygen effect refers to the diminution of many of the changes that are induced in chemical and biological systems by X-rays and gamma rays when irradiation takes place under conditions of relative oxygen deficiency. Viewed in historical perspective, a number of landmarks appear along the way. I think it is sufficient for our purpose simply to recall the early interest and accomplishment in this area. The emphasis in the very early investigations was placed mainly on physiological aspects of oxygen deficiency as related to radiosensitivity. Our interest in the contribution of oxygen to radiation processes has since been very profoundly influenced by growing awareness of the events that are presumed to transpire in irradiated water.

The protective role of hypoxia has been established for a variety of biological materials and for a variety of radiation effects. All radiation reactions are not modified by oxygen lack, but this fact is not inconsistent with our present concepts of action.

In general, the radiation dose required to induce a degree of change in the absence or near absence of oxygen comparable to that produced in air is increased by a factor of about three. It is important to stress that the oxygen effect increases very sharply from levels around zero to about 10 percent oxygen, and then plateaus at levels approaching the physiological or normal ambient tension. The effect appears to be strongly dependent on radiation quality, being greatest with X- and gamma rays, apparently intermediate with cyclotron or fission neutrons, and essentially absent with alpha particles.

The rather parallel influence of oxygen on the behavior of simple aqueous systems and of living systems to radiations suggests that the oxygen effect is perhaps related to modification of the pathways of energy transfer in water. There are certain exceptions to the condition relating oxygen to events in water, but for the most part the data would seem to fit such an interpretation. We must keep in mind, however, that there are other possibilities and that we cannot put all of our eggs in one basket.

It is believed that irradiated water tends to decompose into free radicals if conditions are suitable. The primary products are free hydrogen atoms and free hydroxyl radicals. If oxygen is present in the medium, the

hydrogen atoms may be converted to another reactive intermediate, the hydroperoxyl radical. This in turn can be degraded to hydrogen peroxide. There are several schemes for this.

One effect of oxygen in irradiated water is to produce an oxidizing agent at the expense of free hydrogen atoms. However, there is another influence of oxygen. Owing to the reduction in hydrogen atom concentration, the presence of oxygen would be expected to decrease the back reaction of OH radicals to water and, therefore, to facilitate their reaction with other substances in the environment.

According to this scheme, the presence of oxygen could theoretically increase the oxidizing potency of irradiated water by a factor of about four (OH, HO_2, H_2O_2). For what it is worth we may recall that the x-radiation yield for many biological effects is decreased by a factor of approximately 3 under hypoxic conditions. This then corresponds roughly to the presumed change in reactive species formed in x-irradiated water in the presence and absence of oxygen.

It seems to me important from the considerations I have presented so far to emphasize that the oxygen effect appears to be due for the most part to a change in the biological effectiveness of the radiation rather than to a basic change in the sensitivity of the responding system. In other words, we may be changing the way in which the radiation energy is dissipated, rather than conferring some change upon the cell which makes it inherently more resistant or more sensitive to irradiation.

So much for the background of the oxygen effect. I should like now to turn very briefly to some of the so-called oxygen binding agents. A number of chemical and pharmacological agents have been used experimentally to modify radiation effects. Perhaps the most thoroughly investigated have been the sulfhydryl compounds and the amines. Although interpretation is complicated, there are a number of parallels to the oxygen effect, and it would appear that in many instances (not in all) a similar mechanism may prevail. This is almost certainly the case with the methemoglobin-producing agents, sodium nitrite, and paraaminopropriophenone. Many of the effects of agents such as cysteine, the sulfhydryl containing amino acids, and beta-mercaptoethylamine which is decarboxylated cysteine, also seem to be related to oxygen availability. It is not within the scope of this discussion to consider these matters in detail. It may be said, however, that departures from uniformity of protection can in many instances be attributed to the distribution and biological fate of the various substances.

The oxygen effect and chemical protection have been mainly of theoretical interest, providing further insight to our general understanding. For a number of reasons the agents that have been used experimentally would not seem to provide a practical prophylaxis for radiation effects in man. In the first place, it is necessary to achieve severe hypoxia or to use near toxic amounts of these various chemicals in order to reduce the radiation effect appreciably. There is also the requirement that such agents or conditions must be present during the irradiation and hence must be given shortly before an anticipated exposure.

From the point of view of radiation therapy we may inquire about the advisability of employing agents that manifest a rather general action against radiation effects to minimize, for example, clinical radiation sickness. Unless selective protection can be achieved, the purpose of the therapy might well be defeated, in that the area to be treated may also be more resistant.

Nature, however, may have provided an application of potential significance, which is the basis for the suggestion by Gray that high oxygen tension may be a useful adjunct in radiotherapy of tumors. I should like now to examine briefly the theoretical basis for the proposal made by Dr. Gray, which has now led to some clinical studies.

First, an increase in oxygen tension above the physiological level has a relatively small effect on the response of normal tissues to radiation. Second, many tumors are hypoxic relative to normal tissues. From this it follows that there may be a differential gain in sensitivity in favor of the tumor when there is a general increase in oxygen tension at the time of irradiation.

A limiting factor to the first condition is, of course, oxygen poisoning, and the possibility that the effects of very high oxygen tensions might actually synergize with those of irradiation. Continuous administration of pure oxygen even at atmospheric pressures may lead to pulmonary damage within a few hours in some individuals. Inhalation of oxygen under pressures in excess of several atmospheres may lead to generalized convulsions within minutes to hours. Toxic effects in mice breathing oxygen at 5 to 6 atmospheres pressure appear to synergize with those resulting from high doses of X-rays. These considerations point to an upper limit for the sort of application proposed by Gray.[1, 2]

The anoxia of tumors relative to normal tissue is apparently a consequence of their inadequate and/or abnormal vascularization. This is perhaps more obvious in rapidly growing tumors.

Critical factors in oxygen delivery to tissue are the amount of oxygen physically dissolved in the blood, and the volume and rate of blood flow. These two factors establish the oxygen gradient from blood to tissue. Although hemoglobin is nearly completely saturated at 20 percent oxygen, the amount of oxygen in solution does vary directly with the oxygen tension in the inspired air. Hence, breathing pure oxygen one will have five times as much oxygen in solution in the blood as when one is breathing air. In principle, this would increase the mean amount of oxygen even in a tissue that is inadequately vascularized because of a more favorable gradient for diffusion.

This conclusion, however, presupposes that there are no changes in local blood flow as a result of increased oxygen tension and that the oxygen consumption of the tissue cells is unchanged. If oxygen utilization by a solid tumor is increased when the partial pressure of oxygen in blood is increased, this would be most obvious in those hypoxic cells that are closest to a vascular supply. More distant cells might show very little change in their oxygen content, unless the gradient were sufficient to satisfy the requirements of

the closer hypoxic cells, or their oxygen consumption were diminished by some concomitant treatment. This means that there might be no change or only a very minimal change in mean tumor oxygen tension when, for example, pure oxygen is breathed because only a few cells would have access to the increased oxygen. Because of the nature of the relationship between oxygen and the radiation response, however, I would remind you of the fact that a very small over-all change in the mean tumor oxygen tension could result in a definite increase in effect if the tumor were initially sufficiently anoxic relative to the normal tissues.

KAPLAN: May I interrupt here? I think the last point that you made is an important one. Let's assume that you provide enough oxygen to get to a hypoxic tumor cell in significant quantity. You said that this might change the cell's utilization of oxygen. The net result might be that there would be no change in the intracellular concentration of dissolved oxygen in this tumor cell, and the point I want to reenforce is that as far as radiation effect is concerned, it does not matter how much oxygen the cell is using.

PATT: Yes. I am glad you mentioned that. I was going to point out that one way of selectively increasing the oxygen content further would be to use some concomitant treatment which would depress tumor oxygen consumption. Agents such as cyanide have been used in animal tumors for this purpose. There may be less objectionable materials which might accomplish the same thing and we ought to think about the possibility of this sort of adjunct to oxygen as a means of increasing tumor oxygen tension.

CURTIS: What you are saying, if I get it correctly, is merely that you might not get as much of an effect as you would hope to get, but that does not mean by any means that you would get no effect because of this phenomenon that you have indicated.

PATT: I am merely pointing out some of the physiological variables and limitations. Attempts have been made to measure the oxygen tension within solid tumors of varying sizes and, in fact, a report on this was presented at the cancer meeting in Chicago last month.[3] As I recall, there was little change in mean tumor oxygen tension when pure oxygen was breathed. With oxygen under pressure the situation might be more favorable.

QUASTLER: I want to get back to Dr. Curtis' point for a moment. It makes a big difference whether all the measurements you take will simply give you not quite as great an oxygen effect as the theoretical maximum, or whether you can actually get a paradoxical effect, with a fall in oxygen tension in the cell. Can you think of any secondary measure which would actually have a paradoxical and inverse effect?

PATT: I am not sure that I understand the question.

QUASTLER: You want to raise the oxygen content in the cells. You do it by increasing the oxygen that goes into the lungs. You point out that the increase is not necessarily going to be as big as you would hope for.

PATT: In the tumor.

QUASTLER: Is it possible that you could actually decrease the oxygen tension in the tumor cells by some radical change in oxygen consumption?

KAPLAN: Dr. Quastler is wondering whether you could actually stimulate an over-compensatory increase in oxygen utilization such that the tumor cell would wind up with less oxygen than it had before you started to increase the blood oxygen concentration. I cannot see how that could happen, because the same mechanism that would be stimulated by increased oxygen availability would automatically shut off as soon as the oxygen level fell again.

QUASTLER: There is a big difference between whether it can do damage or merely cannot do as much benefit as you hope for.

BOND: You said earlier that it requires a certain degree of hypoxia to produce this oxygen effect.

PATT: I was referring to the protective effect.

KAPLAN: What about the question of blood flow?

PATT: Blood flow is, of course, a relevant factor. All I can say concerning this point is that inhalation of pure oxygen or oxygen under 2 to 3 atmospheres pressure decreases the blood flow through the brain. I am not aware of any data, although I confess I have not exhaustively surveyed the literature, which would bear on this question as far as tumor blood flow is concerned.

KAPLAN: Gray has made the point that in many tumors as the tissue growth pressure increases there will be stasis in many of the tumor capillaries. Wherever there is stasis the diffusion gradient goes up very sharply; therefore, one's chances of improving the oxygenation of tumor cells at the venous end of a capillary would diminish greatly under those circumstances.

BOND: Apropos of this point, does not the tumor have to be extremely hypoxic before you will get such an effect?

PATT: The tumor would have to be hypoxic relative to normal tissue. I could go on and tell you about the animal experiments and clinical trials; perhaps we could take up your question in the general discussion later.

Gray has reported some work done by Oliver Scott in his laboratory. Scott used a relatively cellular tumor. He inoculated tumor cells into the flanks of mice, where they grew as solid neoplasms. Scott found that the efficiency of a single dose of X-rays for tumor regression was increased by a factor of about 1.5 when the tumor-bearing mice were breathing oxygen at 1 atmosphere. In other words, 1,000 r with mice breathing pure oxygen was about equivalent to 1,500 r when the mice were breathing air. In contrast to this factor of 1.5 for tumors, Scott observed that the skin reaction was increased by a factor of about 1.2 when mice were breathing oxygen at 1 atmosphere.

Hultborn and Forssberg[4] have reported observations on four cases in which skin tumors were treated with X-rays while the patients inhaled pure oxygen. What they did essentially was to map out the tumors, divide them into two parts, and irradiate one part while the patient was breathing oxygen and the other while the patient was breathing air. They reported that the breathing of pure oxygen appeared to amplify the radiation effect somewhat, particularly when the tumors were small, of the order of a centimeter in diameter.

This was not the case in the one instance where a large tumor was treated. This is only one case, but it may be important because Gray makes the point that large mouse tumors also responded less favorably in Scott's work. The factor of 1.5 was observed only when small tumors were irradiated. The sizes were not indicated.

Churchill-Davidson and his associates[5,6] have reported a salutary effect in several patients breathing oxygen at three atmospheres pressure during tumor irradiation. This work was performed on anesthetized patients, with very careful attention to the development of compression and decompression. There are a number of difficulties in the interpretation of their results, because of the fact that they did not control a number of variables such as anesthesia. When their patients were breathing air they were not anesthetized and they did not receive all the premedication that the same patients received when they were treated while under three atmospheres of oxygen. Second, the order and time of treatment with and without oxygen were apparently not controlled. They treated five patients first with oxygen under three atmospheres, and then several days later they treated other areas while the patients were breathing air. In other cases they first treated their patients while the patients were breathing air, and then one hour later they began to premedicate, to anesthetize, and to use oxygen under pressure. As I read the description of their results, I concluded that the effects they observed were quite indefinite, and I frankly did not quite understand why they thought their results were encouraging. I think that we should give careful consideration to oxygen tension as a possible factor in radiation therapy. I hope that these introductory remarks will provide a basis for general discussion of the problem.

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3. Clinical Manifestations of the Oxygen Effect

James J. Nickson

In view of having opened up the discussion on the clinical experiments that were initiated by Dr. Gray's observations with animal tumors, we might continue with that. I would certainly agree with Dr. Patt's conclusion that, on the basis of information presented in the Lancet paper, there is little to get excited about. Since that time the group has carried on their experiment despite widespread criticism of its design. They feel, and with some justification, that they are conducting a pilot experiment. If the initial results are encouraging, then a more formal experimental design can be set up, with randomization of patients and adequate control of the many variables concerned.

Since their preliminary report of December 1956, they have treated a total of 10 patients with carcinoma of the bronchus, 8 patients with cancer of the larynx, 11 patients with cancer of the oropharynx (that is, tongue, tonsil, and pharynx), and 6 miscellaneous patients.

FLETCHER: What kinds of larynx cancers?

NICKSON: Extrinsic for the most part. For lung cancer there seems to be nothing much. Eight of the 10 patients have died with the disease present in the area of treatment.

KOHN: Could you say a word about the radiation doses and the degree of fractionation employed?

NICKSON: In general the schedule called for 1,000 r at a single sitting, and at 7 or 8 days thereafter another 1,000 r. This is a sharp departure from the schedule we normally use in radiation therapy. Arbitrarily to say that the changes relate only or primarily to oxygen is a risky statement.

JOHNS: In the first experiment they treated half of the tumor and now is this the whole tumor?

NICKSON: They are treating the whole tumor. The judgment as to effect in terms of comparison with earlier treatments is entirely a value judgment at this time, and you can accept or reject their value judgment as you see fit.

In carcinoma of the larynx they have treated eight cases of which at the present time four are alive without growth, two have died and had no residual growth at autopsy, and two have died with growth present. I will go into some detail on the larynx and tongue because I think it is the most interesting group.

The survival times are short. The longest is of the order of one year.

On the other hand, if you consider the source of the material that they were treating and the extent of the cancers present, some of the responses are interesting. One larynx is described as a large ulcerative lesion extending from the piriform fossa to involve the base of the tongue, an infected mass 5 x 5 cm. The histology was squamous cell carcinoma. The minimum tumor dose was 2,300 r in a week.

Another patient with a left piriform fossa primary with nodes in the left neck received 2, 300 r. There was complete regression of the primary and nodes. The patient died of liver metastases six and one-half months later from another primary with no evidence of persistence of the piriform fossa primary.

Another patient had an ulcerative supraglottic lesion on the left side of the pharynx treated with 2, 000 r in one week. Apparently complete initial regression resulted, with recurrence four months later.

These are all very large lesions which, in the judgment of the responsible physician, were not suitable for classical radiation treatment. The general pattern was that of complete regression of the primary growth. The time of follow-up is too short for final evaluation.

In the oropharynx area there were 11 cases; 4 tongue, 2 tonsils, 1 nasopharynx, 1 soft palate, 1 lateral pharyngeal wall. Alive, with no evidence of growth, 8; alive with growth present, 1; died with no growth present, 1; died with growth present, 1. The time is short; the lesions were large.

I think most of us who have dealt with this sort of thing would agree that the initial response is of interest.

In the miscellaneous group, they have been particularly interested in patients with sarcoma. They have had, I think, two in which the response has been good, one of which has been followed for 10 months with no evidence of recurrence after 2, 000 r.

KAPLAN: Isn't it true that in this category particularly they have run into a number of cases of very serious necrosis?

NICKSON: I have just had a letter from Oliver Scott in which he says the necrosis in the cartilage in these patients has been impressive. I suspect it is also true that the majority of the cases, judging from the description of the primary lesion, probably have cartilaginous invasion. No comment is made about the other normal tissue reactions.

KLIGERMAN: In their original report, in which they divided the field, several of the cases showed a greater skin reaction when the oxygen was used.

BOND: Dr. Nickson, you say this dosage schedule is not what you would usually use?

NICKSON: We don't. This is something that has been called very vigorously to their attention by a number of observers, that logically they ought to be treating patients without oxygen with the same 1,000 r times 2 with a one-week interval. They ought to be doing it without oxygen but doing everything else in the same way. As Dr. Patt just mentioned, a very complex procedure is employed and the controls will have to be elaborate. Until they have adequate preliminary evidence they prefer, since the technique is so difficult and complex, to concentrate on the question: do we have an effect or not? If the consensus is yes, then a much more elaborate experimental design, which is going to be very costly to execute, is warranted.

Now I am aware of the holes in this argument, and I think they are also. For example, from the pharmacological point of view there could be a positive O_2 effect which they are missing with their pilot study. On the other hand, this is the way they have chosen to proceed. If the preliminary data is highly encouraging then they will do a formal experiment.

Their preliminary data are interesting in terms of the immediate tumor response. I know of no experience of treating primaries in the head and neck region with anything that resembles these time-dose schedules. Does anyone in this room?

CHAMBERLAIN: For late palliation, yes, it has been done, and you can get regression of the tumor, but not a complete cleanup of the primary.

NICKSON: Did they do anything resembling this in the '20s in Germany?

QUASTLER: No, not that high. This is more than an erythema dose to the tumor less than a week apart.

NICKSON: Some of the regressions have gone on for 10 to 12 months. The proportion of regression I think is impressive, certainly to those of us who attempt palliative irradiation.

FLETCHER: Do you have any idea of the size of the portals through the whole neck?

NICKSON: They do not give information on the size of the portals. It is their custom, I know from talking to them, to cover the entire known tumor-bearing area. That means large portals.

FLETCHER: I am amazed that those cases behaved so nicely.

FRIEDMAN: I recall a group of patients with cancers of the mouth and larynx who were treated with 3,500 r in five days. Most of the region sloughed out, and several of the patients died from overwhelming necrosis.

NICKSON: One of their patients died with extensive radionecrosis and no residual carcinoma. So this is a hazard in this group of patients as well.

They have not gone to further fractionation of the doses because of the

great complexity of this form of treatment. It takes four hours to do one treatment.

FLETCHER: But it should be no problem to do that part of the experiment. It is simple.

NICKSON: They have not done it yet.

KOHN: I want to add that there is one other factor that comes in here. If we consider that much more fractionation would normally be given, it would seem that that would tend to improve their results, not only because fractionation, presumably, in general is better, but also physiologically I should think it would make the large tumor more amenable to this type of therapy. As Dr. Patt pointed out before, if you raise the oxygen tension you may not get as good a penetration of the oxygen all the way through the tumor as you want. But if you treated it a few times and peeled off some of these cells, the thickness of the tumor would be reduced; therefore, as the fractionated treatment proceeds you get down inside the tumor and consequently you expect a better therapeutic result.

NICKSON: The point that Dr. Kohn raises has been thought of and certainly is a possibility. It is a balance of these multiple factors as to whether you would gain or lose by fractionation.

KAPLAN: You could argue that for the control series the tumor would be shrinking, and the need for increasing oxygen would be less and less as time goes on. More and more of the residual tumor cells would be close to the blood supply, even though the blood supply is increasingly damaged.

KOHN: I am just adding this as an additional physiological factor that enters here, that they may be able to improve their results by changing the conditions of fractionation. This is not necessarily the best possible test.

KLIGERMAN: This isn't quite as large as it appears. It is a large individual dose, but it is not as large as it looks over seven days.

NICKSON: It could be argued, why bother to irradiate the patient at three atmospheres of O_2 pressure? If you accept the Gray curve which I have attempted to reproduce here, with the tumor cell on the average being depicted at this level of oxygenation and the normal cell here, why bother to go to three atmospheres? Above one atmosphere you are on a plateau, if the animal data has any meaning. Therefore, you are not gaining much by moving up above about one atmosphere, and certainly it is infinitely easier technically to irradiate at that pressure.

PATT: I think the important consideration is that the optimal pressure of oxygen will probably depend on such factors as the size and nature of the tumor, its vascularization, its rate of oxygen utilization, etc.

NICKSON: Many of these things are almost unknowables. That is one of the difficulties. You can argue from the other side of the picture that unless

you have some way of knowing the pO_2 around the cell, perhaps you had better not bother with work in this area at all, which is a pretty pessimistic way to look at it. You can argue that unless you know this, and if you get a spectrum of responses, you will be unable to interpret those responses. The reason for a poor response may be something that has nothing to do with the amount of oxygen going to the alveoli, but the amount going from the vascular bed to the tumor cell. It is the oxygen in the tumor cell that is the relevant factor, as far as we know.

JOHNS: Is there any effect of oxygen going directly to these tumors rather than to the blood supply or is this a very small effect?

NICKSON: How would you get it in directly?

CURTIS: Through the skin.

HIMMELSBACH: Intraarterially would be one way.

NICKSON: That is still through the vascular bed. This has been thought about and no one has been smart enough to find a way to get the oxygen in that does not involve the vascular bed. It would be pleasant if we could, but we don't know how.

About a year ago Thomlinson and Gray published a paper[1] in which they examined the situation in human squamous cell carcinoma of the lung. These tumors grow in rods with the vascular supply on the periphery of the rod. No vessels were observed within the column of the tumor cells. Put it this way: they looked for tumors in which these conditions were satisfied; then they made some assumptions. First that the radiation response was a function of oxygen pressure; second that there was an O_2 gradient in the tissue, as Dr. Patt has discussed; and third they assumed (and they point out that this is a very arbitrary assumption based on work on normal cells) that the consumption of oxygen in microliters per hour per milligram of tissue is about 0.5.

They then looked to see the distance from a capillary to the beginning of necrosis within the cylinder of tumor cells. The assumption was made that necrosis began when the oxygen tension was at zero. In other words, if there was no oxygen the tumor cell died. Theoretical calculations, based on the earlier assumptions, would indicate that about 160 μ from the nearest capillary the oxygen tension ought to be approximately zero. Inspection of a large number of these cylinders showed that no tumor column greater than 200 μ in radius was without necrosis, and that no tumor column whose radius was less than 160 μ had necrosis. In other words, if pO_2 is plotted against radius in μ, at around 160 to 170 μ, the probability of death of the cell rapidly approaches one and at 200 μ it is one.

This setting is compatible with the assumption that as the oxygen gradient is falling, cells that are in, say, the 100 to 160 μ range are under a very definitely diminished oxygen pressure. If you then can increase the level at which oxygen is coming into this system you presumably raise the whole curve.

Dr. Patt has pointed out the difficulties of doing this. The Thomlinson-Gray argument is circumstantial, but it is a rather pretty one. I believe the authors point out that this sort of situation could also be a reflection of a metabolite that is diffusing out. This is another alternative explanation.

Other bits and pieces of ancillary clinical evidence can be felt to exist. If you recall the serial biopsy study that Dr. Glucksmann[2] has been doing at Cambridge, he has frequently made the comment that the cells which show irradiation damage earliest are those cells which are next to the stroma.

PATT: There are several lines of evidence in support of this. I should like to recall some of the work that was done several years ago by Vincent Hall, in which he showed that the radiation response in tumor fragments, either in vitro or in vivo, was related to their size. He showed also that cyanide would increase the radiosensitivity of the tumor fragment. This, however, did not occur in the absence of oxygen. In the tumor fragment the more centrally located cells are probably anoxic relative to the more peripheral cells, the larger the fragment, the greater the difference between center and periphery. This seems to be a reasonable explanation for the relationship between tumor size and radiation response. The cyanide effect can be interpreted on the basis of an inhibition of respiration, which permits diffusion of additional oxygen into the more centrally located cells.

NICKSON: I would like now to turn to clinical studies of skin reactions. Historically there have been statements, usually without numbers attached to them, regarding clinical observations of situations in which the effect was almost always erythema, because the skin is the thing that the radiologists see. These effects can be summarized as occurring in two directions: those things which decrease sensitivity, and those things which increase sensitivity.

The summaries below give the effects of agents studied and the direction of the effect. The number refers to the author responsible for the statement. These are listed in the accompanying bibliography.

Decrease in Sensitivity

Physical Effects:

A. Compression decreases degree of erythema to given dose, no percentage stated.[3,8] Epilation dose increased if irradiated area compressed.[7]

B. Cold + compression decrease degree erythema, no percentage stated.[3]

Physiological Effects:

A. Edema decreases erythema if noninflammatory.[8,12]

Increase in Sensitivity

Physiological Effects:

A. Hyperthyroidism increases effect. No numbers.[11]

B. Edema with infection increases erythema. No numbers.[12]

C. Denervation of skin increases "sensitivity to radiation."[4]

Physical Agents:

A. UV: 200 r + 1/2 unit UV = 300 r + no UV.[5]
   Increased sensitivity.[6]

B. Heat
   Need 30 to 40 percent less ionizing radiation for some degree erythema if heat also applied.
   No quantity named for radiations.[8]
   If skin at 112° F., 1/3 to 1/2 amount of ionizing radiation necessary to give full erythema.[9]
   Other authors state increased sensitivity. No numbers given.

Chemical Agents:

A. Tincture of iodine on the skin during radiation increases sensitivity. No numbers given.[6]

GARCIA: May I mention some evidence on the effect of anemia? There were two groups of patients with carcinoma of the cervix that we handled in 1945 to 1949. There were about 220 cases in each group, one with a normal blood count and one showing anemia, which we specified as a red cell count of less than 4 million and hematocrit values of less than 45. Among the patients who had normal blood findings the recovery rate was 52 percent. Among the patients who had anemia the recovery rate was 33 percent. This difference was significant because of the size of the two samples.

NICKSON: And everything else in the two samples--

GARCIA: Corrected for all the differences in composition the differences remained.

NICKSON: I am personally convinced that the question of nutrition is clinically important, but I have been unable to find any work which would document this impression.

BOND: Did I understand you correctly? Did you say that the erythema was increased?

PATT: Yes, the skin reaction was apparently increased when the animals breathed pure oxygen.

BOND: I should like to know to what degree this comes into the picture that is observed here. If it happened in the skin it presumably would happen in the deeper normal tissue also.

NICKSON: Are you referring to the clinical observations?

BOND: Yes. Haven't you offset your benefits?

NICKSON: What you are dealing with is a ratio.

BOND: That is what I am asking. I mean you definitely have increased the effect in the normal tissue and this tissue affects your ratio.

NICKSON: Well, clearly, if you merely shift the levels at which your ratios occur you have gained nothing. What you are attempting to do is to change the ratios.

PATT: In Scott's experiments the tumor required 500 r less for the same response when the mouse was breathing pure oxygen instead of air. The skin required 200 r less. Thus, the factor for skin was 1.2, for tumor, 1.5. It is the differential effect that is important.

DICKSON: This differential depends on the degree of vascularity of the tumor; so you must have a vascular tumor for this to apply.

PATT: It depends upon other factors as well.

JOHNS: Would the speaker care to say something about experiments that are going on where the patient breathes pure oxygen at atmospheric pressure? I know that Gray is doing that experiment. Whether he has published or said anything about it, I don't know.

KAPLAN: Do you know about any others?

JOHNS: I know that Gray has been doing an experiment for over a year, treating alternate patients with oxygen for about 40 minutes before treatment starts. In other words, keeping everything the same and treating the patient by the toss of a coin. And I know that Dr. Watson, in Saskatoon, has been using oxygen for about three years, but they hadn't reached any conclusions when I left. I know that this has also been done in Hamilton and Halifax.

CARPENDER: Isn't Lanier doing it in Colorado?

NICKSON: I don't know.

DICKSON: The latest I have from Mt. Vernon is that Gray is doing this, but they are not very impressed and I think they are giving it up.

KAPLAN: So far we have been addressing most of our remarks to the relative hypoxia of the tumor cells as contrasted with the normal tissues, but I just wonder about the limiting condition of the high oxygen toxicity and how serious this might be in its own right. Does anyone have any information about this?

NICKSON: I think it is of interest that this group apparently has not recognized any oxygen toxicity.

PATT: Do you mean the Churchill-Davidson group? Of course, their patients were anesthetized, which would tend to diminish toxic effects such as convulsions. Moreover they gave a single treatment. If one breathes pure oxygen for a few hours and repeats this on a fractionation schedule, I think it could pose a problem.

NICKSON: I suppose it is worth mentioning that with tumors on

extremities the use of a tourniquet induces a hypoxic state, giving at 1:1 tumor/normal ratio instead of a theoretical ratio which protects the tumor cell.

FRIEDMAN: It would seem to me that this experiment should be designed to use one type of lesion. An ideal one is multiple recurrent chest wall nodules from breast cancer. With one anesthesia you could give similar doses to three or four lesions, and then with oxygen the same dose to three or four other lesions. This procedure reduces the number of variables as compared with treating individual patients as done by Churchill-Davidson. Even treating multiple chest wall nodules in the same patient in the same type of tumor bed, there is still considerable variation in the response of each nodule, so that you want several at each dose level. Another suitable lesion is melanoma with multiple skin metastases in a patient in fairly good general condition.

CANTRIL: The most ideal patients are those with multiple epitheliomata.

J. R. ANDREWS: There is still another kind of case which has not been mentioned. That, of course, is the radiosensitive tumor with bilateral, more or less symmetrical involvement of the lymph nodes, or the malignant lymphoma with bilateral, more or less symmetrical inguinal, axillary, or cervical nodes. This is an easy experiment to run. It is an experiment which does not cost any money and that can be performed where appropriate clinical material is available. We have attempted this in a few cases in a very preliminary fashion taking a dose somewhat short of that required to produce a distinct response, radiating one area under ambient conditions, and the symmetrical opposite side under conditions of inhaling oxygen at atmospheric pressure. We have not seen any difference which would encourage us to pursue this in a definitive fashion, but it does represent a kind of experiment.

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4. Comments on the Design of a Clinical High Oxygen Experiment

Lincoln E. Moses

I think that experiments of the type where one side of a person or one side of a lesion can be subjected to one treatment and the other to another treatment may be extremely useful to get information about doses, mechanisms, hopeful leads, etc., but for any clinical trial that is done for the purpose of establishing the effectiveness of a method of therapy, I think it would be a mistake to use this approach. The reason for this shocking statement is that since it is an extremely inconvenient method of therapy it probably would never be a serious contender as a routine technique for palliative therapy. Thus the relevant endpoint would appear to be survival, and clearly if one side of the patient has been treated one way and the other another way, whether he survives or he fails to survive, you are unable to say much about which treatment was better.

The second comment I have is also related to the question of adequacy of controls. Consider the question of controls for anesthesia. Let us suppose we have set up several kinds of control for the pressure chamber with anesthesia, three atmospheres of pressure, and four hours of treatment. Finally we come to the question: should we use the control where the only thing they get is the anesthesia plus ordinary treatment? I would say, "No, there is no need for this treatment."

Suppose we don't run it; then in principle we will never know whether the anesthesia is what gives positive results. I don't think this is a serious matter. First, I just doubt that possibility. But, second, if anesthesia is the effective agent, fine. So we think it is oxygen and it is really anesthesia. It is an easy matter to check out later if this is the case.

Now a more relevant matter of this sort is the question of 2,000 r given in two shots. Suppose affirmative results are obtained using high oxygen with two doses of 2,000 r. It appears to be a substantial question, at least so far as short-term regression of the tumor is concerned, as to whether this is due to the oxygen or to the technical dosage pattern. The only way to analyze the separate effects on short-term regression would be to run some subjects without high oxygen. But with regard to survival, a similar control could be omitted by making the following assumption, which may be utterly wrong; namely, that the radiological profession already knows that 2,000 r eight days apart is inferior in terms of survival to other fractionation patterns. Then there would be no point in running a control of that type.

NICKSON: The dean of the radiologists is here. Perhaps he can answer the question.

STONE: My answer would be that two doses of 1,000 r a week apart is not a satisfactory method of treatment. If it were I don't know why we would all be such fools as to take six weeks to treat with 200 r a day.

CANTRIL: The closest approach to that is the so-called concentration technique in which the patient might get 5,000 to 6,000 r in 10 to 14 days. I have seen a good many patients who have been so treated. The survival of those patients in general isn't as good as those who have protracted treatment The complications are very much worse.

LAMPE: What about the short-term survival? Weren't there some very nice results?

CANTRIL: I don't think the short-term survivals were quite as good as among those who had greater protraction.

SCOTT: Henry, you may recall that Sherwood Moore back in the late 1930s was giving a single massive dose of from 1,500 to 3,500 r, and I think sometimes even up to 4,000 r, directly to the tumor through an open operative flap. The treatments were given for certain head and neck lesions and also for tumors of the bladder. He obtained a rather surprising immediate response, but the results were disappointing over the long-term.

LAMPE: The whole point is that you might get immediate favorable responses with that sort of thing; if you wait long enough, then one sees the complication rate, the recurrence rate, and all that. I am not too sure that we know what this sort of dose would do without oxygen.

MOSES: There may be people in the room who can give at least a provisional answer as to whether a couple of large doses are effective, if one takes a long-term endpoint, such as survival over a period of years.

CANTRIL: I don't know of any type of therapy that would yield survivors among the types of patients Dr. Nickson described.

NICKSON: Survival for how long? These are all short-term survivals.

CANTRIL: Most of the patients that you described would certainly be dead within a year.

NICKSON: That is true.

FLETCHER: I think that we are all discussing what we are supposed to discuss later. We are really discussing what is the purpose of therapy and how to gauge its efficiency. It is very obvious that there are factors other than the radiotherapy itself that can influence the end results if you are going to speak only of five-year survival. We recently treated a series of far advanced oral cavity cancers, some at 250 kv to about 4,000 r, others with supervoltage to a tumor dose of about 6,000 r in five to six weeks. In those treated at 250 kv, the primary tumor was controlled in only 25 percent of cases. In the group treated with supervoltage, the primary tumor was sterilized in 80 percent, but 11 of those cases were dead at one year.

MOSES: Of what?

FLETCHER: A good many died from old age, bronchial pneumonia, and distant metastases. If you take as a criterion freedom from disease, 80 percent of those who were treated with supervoltage had freedom from trouble at the primary site, which is a considerable increase and even with our small number of cases is significant. However, the number of survivors at one year was about the same in both groups. A good many of the patients were over 70 years of age.

III. FACTORS RELATED TO THE AGENT AND MODE OF TREATMENT

1. Dose-response Studies, Fractionation, and Recovery

Henry I. Kohn

The three topics for this discussion are themselves part of or closely related to a more general topic which the radiation therapist calls dosage, and perhaps it would be well to make dosage itself our primary concern, and then to consider various topics that fall under it.

Dosage implies, as you all know, not only the absorbed dose, but also the distribution of that dose in time and also the distribution of the absorbed dose within the biological object. Many factors enter into a consideration of dosage and we can consider only a few of them.

I might say, by way of historical introduction that, of course, the topic of dosage is an old and honorable one in the practice of radiation therapy, and that our current ideas, it seems to me, are largely based on the influence of the Paris school, particularly the experimental work and the ideas which were put forward in the early '20s. By way of covering the history of the subject, I will not say anything more but will hand out this little bibliography[1-8] in which are listed eight of Regaud's papers, seven of them having been published between 1922 and 1923. I think if you will read their titles you will see that what we have been discussing here was at that time also being discussed, I dare say with equal interest and in much the same way.

You will notice also that the ninth paper in this bibliography is one by Ralston Paterson.[9] If one is seeking a relatively contemporary piece of work, illustrating the very thing that Regaud was talking about 20 years before, this is a very convenient paper.

The first question we might ask ourselves, then, is what can the laboratory offer today in the light of the fact that the general considerations of dosage have been so well developed in the past and are in constant clinical usage? It seems to me that by way of principles the laboratory can offer us very little at the moment. The general principles certainly are pretty well understood. By means of dosage we aim to achieve selective action of the beams we employ; the therapist's principal job is to regulate the dosage in the most effective way.

I should say that in thinking about this I have really been up against a blank wall--to put forward something really new that has not already been said before, and that probably everyone in this room has not gone over many times. I might just illustrate the difficulty for a moment.

Dr. Kallman and I were interested in doing some recovery experiments

with a variety of animals. In these particular experiments a single dose is given on day zero. It happens to be approximately one-half of the LD_{50} dose. At various times thereafter a second dose of radiation is given in order to ascertain the LD_{50} of the animals. These are whole-body exposures. As one allows more time to elapse between giving the first and the second dose, recovery from the first dose occurs (leading to a change in the LD_{50}) and by this means one can estimate in fact the course of recovery. It is obvious that such a recovery factor is an important consideration in all fractionation dosage schedules. Starting with the A/He mouse, (for unirradiated mice) the LD_{50} is 619 r and the time for half recovery is 1.6 days. This experiment is essentially of the same design as the experiment by MacComb and Quimby on the skin.[10]

Coming to the next strain, we have an LD_{50} of 552 r. The half-recovery time is 2.8 days.

The LD_{50} of these mice is not particularly important for our purposes, but the half-recovery times for mice vary from 1.6 to 7.4 days. The 7.4 days determination is taken from the literature. It is one by Paterson and Gilbert.[11] The others we have done, and the experiments have been purposely arranged so as to use quite the same techniques.

FRIEDMAN: Will you redefine your half-recovery time?

KOHN: The half-recovery time under discussion is a measure of the rate of recovery. Injury was induced on day zero by giving the mice a single whole-body exposure of 350 r, and exposure which kills none of the animals. The LD_{50} of these animals was then determined on various days thereafter. In the table[12] the LD_{50} of previously unirradiated mice is 673 r. On day four of the experiment, the LD_{50} was 585 r. We infer that residual injury on day four equalled 673 - 585 = 88 r; 88/350 = 25%. From a graph of such determinations made at appropriate intervals we ascertained the time for 50 percent recovery.

	LD_{50}	% injury
Previously unirradiated CAF_1 mice	673 r	----
After 350 r on day 0		
day 1	415	74
day 2	480	55
day 4	585	25
day 8	681	- 1

When this technique is applied to different strains of mice, you find that you can have large differences in the half-recovery times, depending on the nature of the mice employed. That is the only point I want to make. When you compare the mouse with the rat, you find that the rat in exactly analogous experiments has rather different half-recovery times, and that, furthermore, two kinds of rats can differ remarkably and unquestionably from one another.

ROBBINS: Are the rat experiments yours?

KOHN: One rat experiment was performed by us,[13] and the other is the well-known experiment by Hagen and Simmons,[14] performed at Oak Ridge during the war. Both experiments were carried out in quite the same way. We took pains to make our experiment match theirs. The LD_{50} of these two strains of rats matched rather well. We took pains to check their dosimetry and this agreement is not fortuitous. It is because we did the experiments in the same way and the rats did have comparable sensitivities. All of the results with mice are also strictly comparable because the initial dose was approximately the same in all cases and because the $LD_{50's}$ were approximately the same.

QUASTLER: I question that.

KOHN: In our laboratory we are able to make them the same.

How can you translate such an experiment as this into clinical practice. This is a perfectly good fractionation experiment, but what does it hold for the clinician? He already knows that recovery occurs in fractionated dosage schedules. He knows that patients differ from one another. This experiment is a kind of laboratory exercise demonstrating a number of things which I dare say are fairly well known in the clinic. It is not a series of experiments in which the laboratory is suddenly illuminating clinical practice. When one tries to design experiments in this field, studying the physiology of protraction, fractionation, dosage, etc., one always runs into this same difficulty, that at present it is extremely difficult to do an experiment which contributes some new principle that the clinician does not know about or some new quantitative fact that he can actually translate into his clinical practice. That is the point of these introductory remarks.

These experiments perhaps have one small virtue in that they demonstrate, probably for the first time, that the genetic constitution of various strains within a species does influence their respective rates of recovery. Perhaps this thought is also relevant in the clinic, namely that some of the variation that one sees in patients is not simply due to their varying physical condition or type of disease or past history, but may, in fact, be constitutional. Of course, from the point of view of the clinician such a principle is not of much help, because he would like to have an indicator which allows him to pick out the more sensitive patient from the more resistant patient.

If we recognize this great difficulty in translating laboratory experience at the present time into clinical practice, then it seems to me that progress today in the study of dosage must largely come from study of quantitative clinical data in the clinic itself.

Dr. Friedman, do you disagree with that conclusion?

FRIEDMAN: With part of it.

KOHN: Would you pass a remark?

FRIEDMAN: Studying the recovery factor with respect to whole-body irradiation of the mouse is an expedition which has no relationship to the recovery factor in the irradiation of tumors.

KOHN: But it has something to do with the recovery factor of normal tissue.

FRIEDMAN: What normal tissue? The hemopoietic system? The osseous system? Each has its own recovery rate.

KOHN: All right, now let's turn to some of the clinical data, these initial remarks on laboratory work having met with such overwhelming success.

FRIEDMAN: I am only referring to an endpoint which is remote from the variable that you are introducing.

KOHN: But I think the same remarks could apply actually if one had studied tumors here, that the quantitative translation of fractionation schedules for tumors in animals is an extremely difficult and perhaps not practical thing for clinical practice. You already know that you have to fractionate the dosage. You already know this is your selective tool. I don't see that it would prove much if I put a squamous carcinoma into rats and then determined the best fractionation schedule for that tumor--once per day for five days or once a week for five weeks--because you know this. Your clinical experience actually is a better guide, I personally believe, for the clinical treatment of squamous cell carcinoma than is my experience with the rat.

FRIEDMAN: Yes, but we must remove our fractionation schedule from the realm of empiricism and some of us are trying to do this, with marked lack of success.

KALLMAN: You are not as far apart as one might think, if you believe the conclusions recently drawn by Mole, in his most recent paper,[15] where the recovery rate of animals subjected to whole-body irradiation is about the same as Strandqvist's rates.

KOHN: We are going to come to those rates later.

I have put on a chart a prescription for treatment. Let's not argue precisely for what tumor. It is presented simply to recall for some of the people not altogether familiar with these factors what they might be. In this particular set of dosage factors the total dose is given as 6,000 rads. The protraction is said to be 39 days. By that is meant the elapsed time from the first treatment until the last treatment is given. The fractionation here is 30. That is, the treatment will be given in 30 doses. The doses are given five times a week, and the fractional dose is equal to 200 rads.

The fractional dose rate in this case is 50 rads per minute. Of course, we now know that this may be complicated by the fact that with the high energy machines the micro dose rate, if you wish to think of it that way, may be

1,000 times what the macro dose rate appears to be and this might, indeed, be an important consideration.

Finally, there is the volume dose. Since we are not specifying a particular patient, we are not going to calculate this out, but it becomes an extremely important consideration, obviously, in the actual treatment of patients.

If we consider the data in the literature that are available for analysis, we find that most of them are concerned with the first three factors listed. They are concerned usually with (a) the total dose as a function of (b) protraction and (c) fractionation, and it is important, I think, to make the point that usually protraction and fractionation are confounded in these analyses--not, of course, because the men making the analyses do not realize this, but because the data are of such a nature that you cannot separate out the influence of these two factors independently of one another.

Usually when a clinical trial on dosage is run, one studies the "clinically effective" dose against the over-all treatment time, and it is customary at present to study treatment time as follows:

You treat, usually, every day. If, therefore, you vary the over-all treatment time you not only vary the protraction, you automatically vary the fractionation. As far as I can make out, at least for X-rays and in terms of current schedules, very little information has been published as to what the effect would be if the protraction were held constant and the fractionation were varied.

WOOD: In the actual design of the experiment or the treatment don't you pick the fractionation and then let the protraction happen as it may because of the fact that you have only five working days a week?

KOHN: Yes, but what I am saying here is that this need not be. You can have protraction for 39 days and decide to treat only three times a week. You would then be studying fractionation independently of protraction. This is my first point, and we cannot discuss it because, as far as I am aware, there is no large body of data from which to discuss it. On the other hand, I am sure that every therapist here, on the basis of his personal experience, must have some opinion on this matter.

CHAMBERLAIN: May I make a comment? Since 1953 the ICRU has requested the reporting of results to include this information. Vincent Collins and I have both been looking through the literature to see the proportion of papers so published and less than one percent of the papers published since that time include this information.

KOHN: This is a problem which it seems to me is worth studying.

FRIEDMAN: A few such studies on clinical material were published from New York. First, your definition of fractionation and protraction is a little different from Coutard's use of the terms.

KOHN: I thought I was being quite consistent here with Regaud and the way he used it.

FRIEDMAN: No, he misused the term.

CANTRIL: Since he invented it, how did he misuse it?

FRIEDMAN: Coutard used the term, protraction, to mean roentgen flux, the r per minute or the number of hours per day during which a patient was being irradiated. The term, fractionation, was used to indicate the amount of the daily dose.

KOHN: In reading Regaud's paper it becomes evident that what he means by protraction is the length of time the radium applicator is on the patient.

FRIEDMAN: The length of time per day.

QUASTLER: With radium it is the same because you put it on once. There is no difference between "dosage flux" and "dosage over total treatment time," as there is with X-rays.

KOHN: It is a matter of what one reads in these seven references here. I think if you will read his Brussels' paper, it will become clear what he meant. He says 8 days and he means 8 days.

Therefore, we get to the point of translating something that was done first with radium plagues to X-ray dosage, where you cannot give the dose continuously because the patient has to go home. So you have to make a decision as to whether protraction is a matter of dosage rate or over-all treatment time.

FRIEDMAN: We are both right. I am referring to Coutard who apparently must have misinterpreted Regaud's French.

CHAMBERLAIN: Acting again as a sales agent for the ICRU may I request that the terms used conform with the ICRU's recommendations on the recording of dose data, in which confusion with these terms was purposely avoided. They are specified as the total number of sessions, the total time over which the sessions are spread, the time distribution of the treatment sessions, and duration of each treatment in either the exposure dose rate or the absorbed dose rate.

KOHN: I am in accord with this.

JOHNS: Another point comes in here. If you want to do a clinical experiment on this problem, you have to keep in mind that usually a tumor is treated through a number of fields, and presumably if you want to treat every day and you want to do the experiment properly, you have to treat each one of these fields every day.

KOHN: I don't see that it is a necessary conclusion that you must treat every field every day in order to do the experiment properly.

CHAMBERLAIN: But you must specify what you are doing.

FLETCHER: Some people have treated three times a week or twice a week, giving the same dose.

KOHN: All I am saying is that I don't know of a paper which reviews this matter in detail and presents some kind of quantitative analysis of such data. Am I correct in saying this? Have I overlooked something?

FRIEDMAN: Just qualitatively, not quantitatively.

KOHN: I might throw in another prejudice. Usually when we think of the results of clinical practice we judge the success of the therapy either in terms of the survival rate or in terms of the morbidity. I think there is also another point to be considered, namely, cost, and it strikes me that it might be possible to maintain the same morbidity and survival, but decrease the cost by a further study of some of these variables. This is great sport, you know; the laboratory man is telling the clinicians what to do.

LAMPE: You have not told us much so far.

KOHN: Touché.

FRIEDMAN: That is unfair. The clinicians are fumbling in these studies and haven't come up with an answer, and the laboratory people at least have some definite data on recovery factors.

KOHN: Let us turn to a consideration of some of the quantitative aspects of the relation between effect and over-all treatment time. As a basis for the discussion, I shall draw on the review written by Andrews and Moody.[16] They compared the results of several studies in which "effective dose" was studied as a function of elapsed time or over-all treatment time. The data on which these analyses were made are summarized in the following slide:

First, in Strandqvist's classical study for tumors of the head region,[17] they were superficial and the over-all treatment time was 1 to 29 days in the 150 or so collected cases which he pulled out.

FLETCHER: Two hundred and seventy-three.

KOHN: I will not leave that unchallenged.

FRIEDMAN: There is Strandqvist's slide. It is 258, I think.

KOHN: I am sorry. Take that slide off, please, before it confounds the issue. If you will read Strandqvist's paper carefully, you will find that Strandqvist for his final curve pulled out of his series the 95 cases which he considered matched, and these 95 cases gave the best fit to the curve that he offered. These cases are the ones that we are going to talk about here and they were treated in an over-all treatment period of 1 to 29 days.

Second, in the data of Hale and Holmes for skin tumors,[18] the over-all treatment time was either 1 or 8 days. These two points were plotted with a point based on Jacobsson's determination of the effective dose for treating tumors of the hypopharynx in 29 days.[19]

Third, the data for radical small field treatments is from Paterson.[20] This is not based on a quantitative analysis of the data but I believe it is Paterson's over-all impression of what the effective doses are for treatment times of 1 to 33 days.

Finally, only in Dr. Garcia's series for carcinoma of the cervix[21] did the treatment times extend beyond 35 days. They ran from 2 to 94 days.

This is the series of four curves drawn by Dr. Andrews and Dr. Moody, based on the four sets of data just mentioned. (Slide) The upper one here is for Strandqvist's data, the next for that of Hale, Holmes, and Jacobsson, etc. The point I want to emphasize about this is that for three or four series the graph is a fitting of points up to 30 days. For one of the series the graph is a fitting of points out to 94 days. The only point I want to make here is that we don't have much information about the relation between effective dose and over-all treatment time beyond 30 to 35 days.

CORNFIELD: I wonder if you could explain the meaning of one point on this graph?

KOHN: Do you mean, for example, this one here?

CORNFIELD: Yes, that one. What does that mean?

KOHN: This graph represents what is considered to be the logarithm of effective treatment dose as a function of log-elapsed time.

GARCIA: May I comment on that. Each one of these lines represents a different degree of effectiveness. The thin lines represent something like 85 percent effective dose schedule, and the one that we happened to prepare represents about a 50 percent degree of effectiveness.

KOHN: I don't want to interrupt but did everyone hear Dr. Garcia's statement? Will you say it louder?

GARCIA: No, you go ahead.

KOHN: His point is that, let us say, in one series the effective dose was defined as that dose which gave 5-year survival in 85 percent of the cases. On the other hand, in another series, the effective dose was that dose which gave a 5-year survival in only 50 percent of the cases. So the endpoints are not strictly the same for the four curves in the graph.

KLIGERMAN: Strandqvist does not give 3,300 r in one day as a single dose but rather 2,250. I don't think there are many therapists who would attempt to give that dose.

FRIEDMAN: Excuse me, I think you have been pointing to Strandqvist's skin necrosis curve.

J. R. ANDREWS: This was modified. For reasons I will not attempt to give now, this is not Strandqvist's original plot. My paper[16] points out what was modified and why. This difference is recognized. This is the important point: the slope is the same.

FRIEDMAN: The slope has been arbitrarily made the same.

GARCIA: Another thing I want to point out is that the dosage schedule was applied to volumes of different dimensions in the three studies.

EVANS: May I ask a question? The selection of a certain dose involves a balance between two factors. If one goes beyond a certain range, one gets too much necrosis. If one goes below that range, one gets too little effect on the tumor. The effective dose is a balance between a good result as far as the tumor is concerned, and a bad result as far as necrosis is concerned. So it is a compromise dose. Am I correct in that as it applies to the skin lesions?

KOHN: Basically it is always a compromise between many factors, but the chief point I am trying to make here, without arguing about all the details, is that the data are not many on which we can arrive at efficient dosage schedules, at least insofar as they are summarized in this manner, and furthermore that there are very few data which go beyond 30 or 35 days. Dr. Andrews and Dr. Moody modified this first part of Strandqvist's curve and, as he says, this was pointed out in their paper. If you want, we can chop off the first day or so here. The fact remains that these curves for four sets of data tend to be parallel, which is indeed a somewhat striking fact. But the other fact is that we don't have much data past 35 days.

I will now make my last point. Let's consider the validity of this type of plot in the case of Dr. Garcia's data. If you will look at the papers that have been handed out, I have plotted Dr. Garcia's data on an arithmetic scale. The ordinate is simply the effective dose for the series, and the abscissa is the over-all treatment time. If you will cover up the first seven points for the first 30 or 35 days of treatment and look at the remaining points on the graph for 35 to 90 days of treatment, I think you will see that a straight line through them with practically no slope is a reasonably good fit. The equation of the line is: Dose (r) = 6, 280 + 2.6 T (days). On the other hand, if you will cover up the last 17 points and look at those for the first 30 to 35 days, you will see that the curve drawn here, which is essentially the curve that Dr. Garcia drew and that Dr. Andrews had in his curve, is likewise a reasonably good fit. Its equation is: Dose (r) = $2,944\ T^{0.197}$.

All I wish to suggest is that taking these data at face value, it may be that the classical Strandqvist plot, which seems to be a reasonably good fit for different kinds of data, perhaps not for 1 day but for from 2 to 35 days, may not apply in the region beyond 35 days. This, of course, is simply speculation.

QUASTLER: I am much impressed by the data that were presented. I am also much impressed by the difficulties of fractionation studies in the laboratory. Clinical studies, of course, are fraught with even more complications. The fact that a set of data can be plotted as a reasonably straight line on some kind of graph paper is nice; it indicates a general trend, but it is not all we need to plan rational fractionation schedules. For instance, let me remind you that there is no law on earth that says that five treatments a week given at 24-hour intervals is better for anybody but the therapist.

In the laboratory we definitely know of cases where two doses separated by an interval of time are as effective as the same two doses given without an intervening interval. We know of many cases where the intervening time interval reduces the effectiveness. We also know of cases where it increases the effectiveness. We have no reason to be quite sure which category is appropriate for a particular tumor. Furthermore, in many of those cases in which a time interval modifies the effectiveness of a response, it also changes the character of the response so that you cannot necessarily reproduce a single-dose effect with divided doses.

If I may, I would like to read into the record some data obtained by Reisner.[22] Reisner's experiment was similar to that of MacComb and Quimby, only he gave various fractions of the erythema dose in the first treatment, and determined how much had to be added one day later to produce the standard erythema reaction. The values he found were: 90% on the first day, 20% on the second; 80% and 35%; 70% and 50%; 60% and 65%; 50% and 80%, etc. Compute the ratio of residual dose over dose applied; it decreases from 80/90 = 0.89 to 15/40 = 0.38. This is far from a constant fraction.

Reisner also established the number of days, including Saturdays and Sundays, you have to administer a given fraction of the erythema dose in order to obtain a response similar to that obtained with 100% given once (100% x 1). He found that 65% x 2, 50% x 3, 40% x 4, 30% x 7, 20% x 12 and 10% x 27 are approximately equally effective. Any attempt to fit those data with a constant exponential recovery coefficient is completely ineffective.

I mentioned those findings just to bring out some of the complications. I believe that many careful experiments are needed. They are very difficult, and one should not cloud the issues by presupposing a particular mechanism of recovery which may not apply.

FLETCHER: I think that Dr. Quastler has said what I was going to say. A good many of these curves are really repeating or accepting what has been solved. If you go back to the Strandqvist curve, actually there are very few cases between 1 and 4 days, and a small number between 14 and 28 days. I happen to know this because I was in England in 1947 when they had just gotten the original monograph from Strandqvist and I saw it and the Curie Foundation data. I was interested in publishing a chapter on radiotherapy of skin lesions and I plotted the Curie Foundation data, which are excellent as far as cure rates are concerned, but they didn't fit the time curves at all. There is a study by Zuppinger which you could have mentioned. In 1932 and 1933, Zuppinger did some studies of fractionation concerning late skin

changes. His curve does flatten out appreciably more after 30 to 40 days in the way that you modified Garcia's curve there. If you extrapolate, the Zuppinger curve taken as another criterion of fractionation effect was decidedly much lower.

Dr. Quastler's final statement is really profound. One starts out too often with the idea of having the exponential curve in mind and doing a good deal of fitting.

KAPLAN: We will hear briefly from Drs. Friedman, Andrews, and Garcia.

FRIEDMAN: In a somewhat roundabout way I am going to corroborate what Dr. Quastler said and show the weakness of all these data, particularly our own data, though it was obtained through a better designed experiment.

The statisticians here would find intolerable the construction of one curve by Strandqvist. For the doses that resulted in failure, he drew a free-hand parabolic curve to represent the information. For the doses that were excessive, he drew another representative parabolic curve. Then he superimposed the two curves and said that the zone between them was an optimal lethal dose zone. If you actually drew the correct curves for the data you would get a weird hieroglyphic.

Strandqvist's patients each had one lesion, and the lesions differed one from another. Some were basal cell epitheliomas, some were squamous cell epitheliomas, some were large, some were small, some were on the face, some were on other parts of the body, and he used different size portals. The fact that he lumped all of these variables together is another weakness of his data.

In our study, we used chest wall nodules from recurrent breast cancer in patients each of whom had from 11 to 35 nodules of approximately the same size, and in the same tumor bed. The size of the irradiation portal was standard. We varied only the dose and the time, and observed the rate and duration of shrinkage of the tumor. This we thought was a better designed experiment.

For example, the first lesion was given a large dose. Successive lesions received gradually reduced doses until we got the smallest dose in a stated time that destroyed the tumor (Figure 1). We disregarded the excessive doses because they would pull the curve upward, and utilized only the minimum tumor lethal dose for each category of over-all treatment time. We constructed iso-effect curves based on the latter points.

Figure 2 shows the collected iso-effect curves from 11 patients with recurrent breast cancer nodules. The lesions in one patient may be very radio resistant and their recovery curve have a certain slope. The lesions in another patient may be more sensitive and the iso-effect curve may have a different slope or a different recovery rate. With this in mind one can appreciate the difficulty in attempting to obtain a reliable recovery curve when

using patients with only one skin cancer, and that being different in type from the next patient's skin cancer.

Although an iso-effect curve for recurrent breast cancer was obtained by more reliable methods than hitherto described, the standard error of the data was too large to permit derivation of a useful mathematical formula for the slope of the curve. In our opinion only the curves of Quimby and MacComb, and of Reisner, based on skin erythema, have any validity. If you have a recovery curve for one patient's breast cancer nodules, it is inaccurate to apply it to all breast cancers. It is even more erroneous to apply Strandvist's skin cancer curve to cancer of the mouth, esophagus or cervix, as has been done.

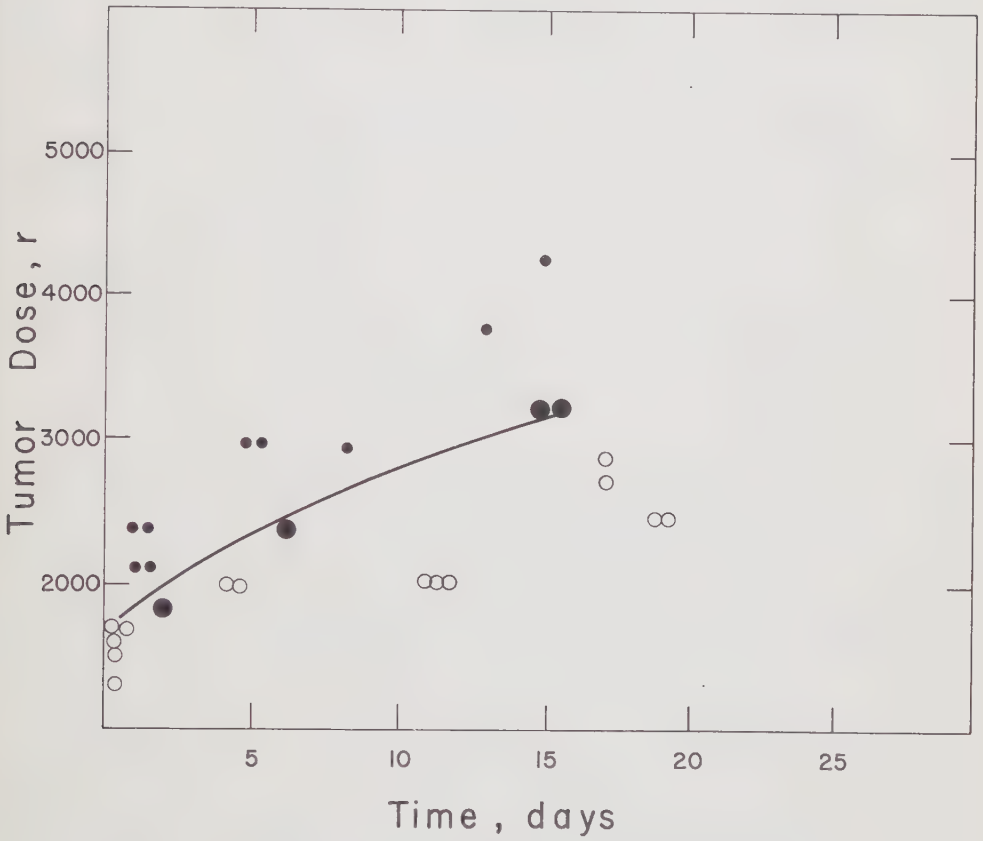

Figure 1. Scatter diagram of doses given to 27 recurrent breast cancer nodules in the same patient. Observations were made six months after irradiation. The circles represent failures. The solid dots are successful doses; the small dots are doses that are probably excessive in amount; the large dots are corroborated minimal tumor lethal doses. The iso-effect curve is based on the latter points and is drawn freehand.

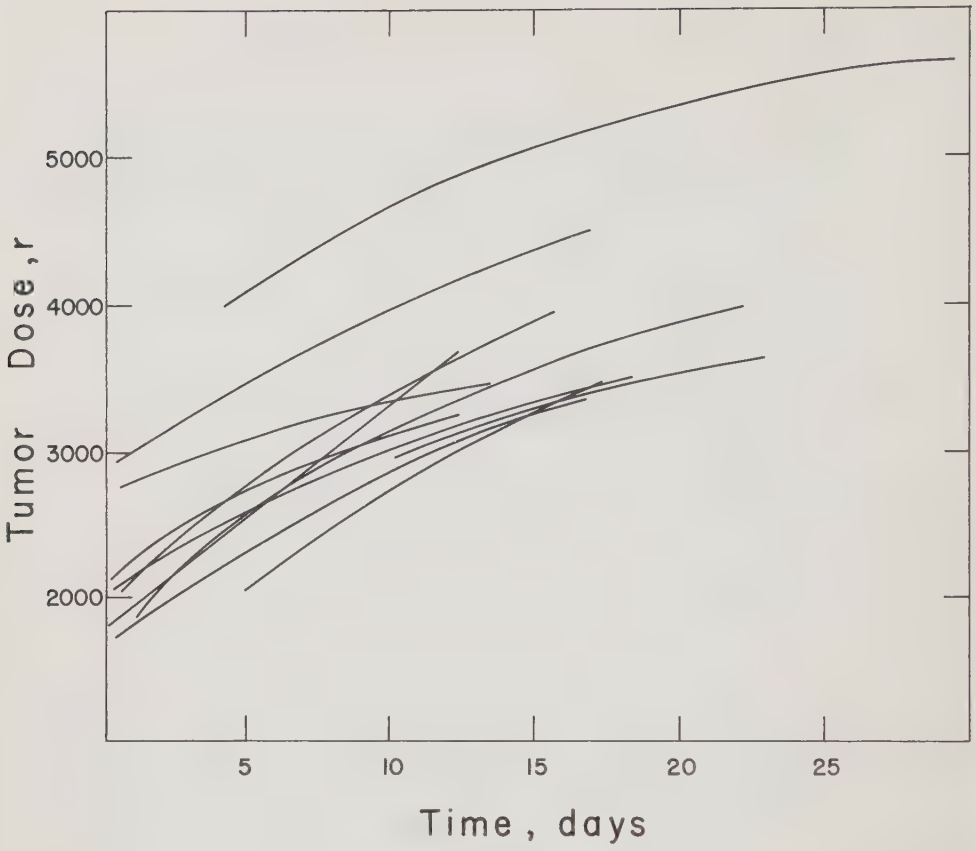

Figure 2. Iso-effect curves, drawn freehand, of 11 patients, each with multiple recurrent breast cancer nodules. One of these curves is illustrated in Figure 1. Note the variation in tumor dose and the different slope (recovery factor) for each patient.

Figure 3 gives our assembled breast data. The best we could do was not to assign a coefficient to represent the rate of recovery, but to divide the data into zones and accept this information as clinically useful but having no statistical validity. For example, doses in Zone A cure a certain percent. In Zone B another percent is cured, and in Zone C a much smaller percent is cured.

In Figure 4 are the data based on 27 lesions of a patient with mycosis fungoides, illustrating once again the method for obtaining corroborated data for minimal tumor lethal dose points in different over-all time periods. This curve was not suitable for other patients' lesions.

In Figure 5 is assembled a number of iso-effect recovery curves based on human biological material. Note the different recovery rates as indicated by the slopes of the curves.

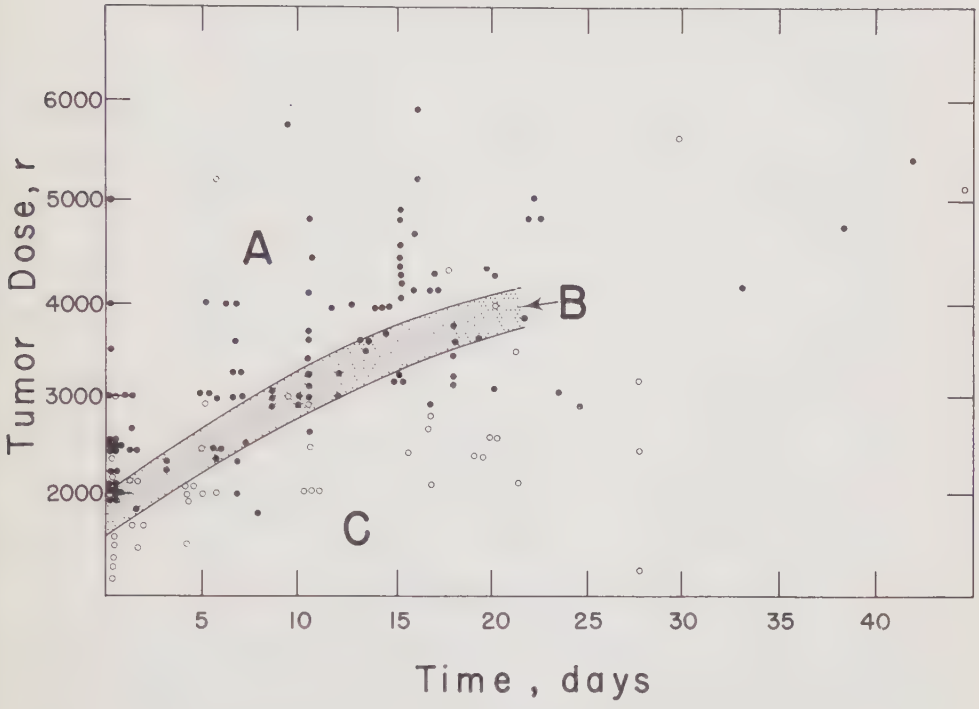

Figure 3. Scatter diagram of all data (142 points) in a breast cancer study. The dots are successful doses; the circles failures. The curve constituting the upper parameter of the shaded zone is the iso-effect recovery curve based on 27 "corroborated minimal tumor lethal doses." It is the closest approximation of an iso-effect recovery curve for recurrent breast cancer yielded by this experiment.

The lower parameter of the shaded zone is based on McWhirter's recommended dose of 3, 750 r in 21 days. There are 90 percent successes from doses in Zone A; 76 percent in Zone B; and 30 percent in Zone C. This spectrum of tumor lethal dose requirements typifies the dosage problem for many tumors and emphasizes the difficulties in obtaining a useful and representative iso-effect recovery curve.

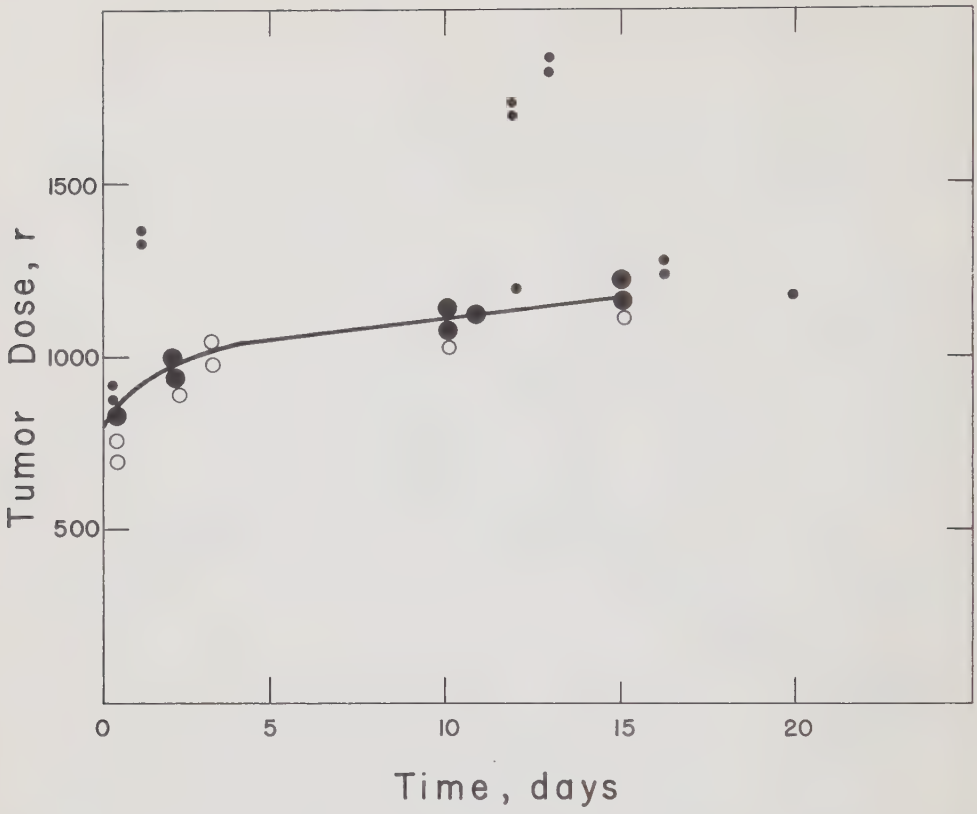

Figure 4. Iso-effect recovery curve based on corroborated minimum tumor lethal doses in a patient with mycosis fungoides, similar to that in Figure 1.

Let us consider the clinical experiments of Zuppinger, and of Manuel Garcia, and Henry Kaplan's brilliant experiment on mouse lymphosarcoma. Different doses were given in different over-all times, ranging from 10 to 50 days. Some time later they evaluated either the death of the patient, or chronic radiodermatitis, or the development of lymphosarcoma in mice. Between the irradiation and the endpoint, thousands of different events could have influenced the eventual outcome. That type of experiment is not too reliable for obtaining data for a recovery curve applicable to the technique of irradiating a tumor. The endpoint should be a phenomenon somewhat closely related in time to the administered treatment. Much more of this work should be done on human material. There are very few tumors that provide multiple nodules. I have the feeling that most of the work will have to be done with animals, and we still will not get much useful quantitative recovery data.

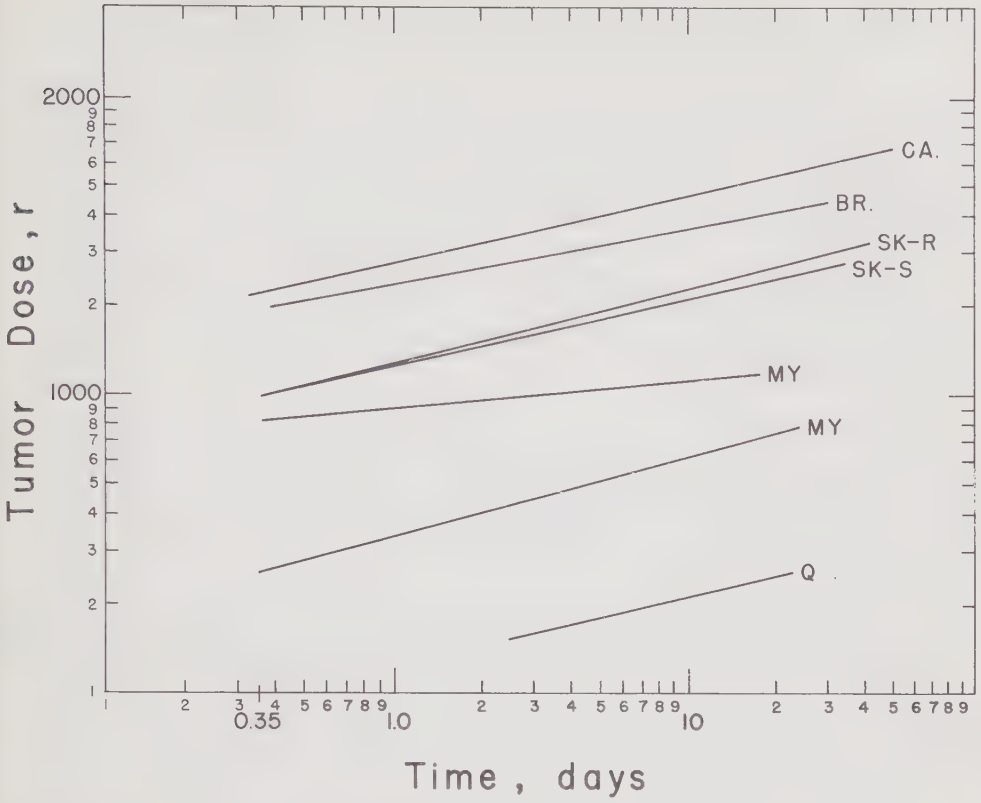

Figure 5. Iso-effect recovery curves for different human tissues. CA-carcinoma of the skin, Strandqvist; BR-skin nodules of locally recurrent breast cancer, Friedman; SK-R-skin erythema, Reisner; SK-S-skin erythema, Strandqvist; MY-mycosis fungoides, Friedman; MY-mycosis fungoides, Friedman; Q-threshold skin erythema, Quimby.

Note the difference in slope for different tissues. The curves for the two cases of mycosis fungoides were accurately obtained on the basis of 27 and 35 lesions respectively for each patient; yet the slope of the curves indicates a marked difference in recovery rate for these similar tumors.

J. R. ANDREWS: I will just report some clinical observations because they have a bearing on this discussion. Without trying to plead a cause, I should like to make two points. One is that if there is not some such relationship as this of dose and time, then we have to conclude, of course, that the dose is very uncritical and we need not pay much attention to it.

The other point I should like to make, I think this is a fair statement, is that it has not been shown that clinical results differ significantly by departing, say, within a range of plus or minus 20 percent from some mean dose value. This has never been demonstrated.

In the last two years I have had the opportunity to treat a very small group of patients for extended periods of time. This is something I thought we could do at the National Institutes of Health, because of the opportunity of keeping patients for extended periods of time without its being an economic hardship on them. As pointed out, there are some data establishing a dose-time relationship to around 30-odd days. I have carried our studies out to somewhere between 90 and 100 days. If the mean value is extended out here to 100 days in a conventional plot, the dosage figure is 8,000 r, which is interesting from one point of view, because many clinics are treating with, say, 8,000 r in around 6 to 8 weeks. In the situation we are talking about here, the conventional dose-time relationship extended to 100 days is 8,000 r. We have carried treatment out between 80 and 100 days with total doses of 8,000 to 10,000 r.

It is interesting to point out that the initial tumor response and the normal tissue reactions are very much what one would expect to see in this dose--time zone down here in the plot, which suggests that there may be some validity to an extension of the plot and to the idea of a more or less parabolic relationship between dose and time.

We have not seen anything that would impress us as significant differential effects between response of tumor and response of normal tissues. This, of course, is an important observation which should be confirmed by the examination of a large mass of additional material, because if we cannot find differential tissue effects in such a manner, then we have to look elsewhere for differential modifiers--either oxygen or pharmacologic agents.

CANTRIL: What types of patients?

J. R. ANDREWS: Oral cavity and oropharynx cases. These were very extensive lesions requiring very large fields. If tissue differential effects were to be seen I think they would be seen under these very adverse conditions.

GARCIA: I should like to point out that some difficulties in interpretation have arisen. When we attempted to define the average dose that brought about recovery in a number of patients with carcinoma of the cervix, we wanted to see if the dosage was alike regardless of the time period. Of course, it became evident that it was not; so we merely took the data and attempted to fit the simplest possible relationship that showed us the time plot of the average dose. It turned out that the patients who had dosages between 95 percent of the value of the curve and 105 percent of the value of the curve gave 50 percent survival. Had we used a different dosage schedule we would have obtained another curve that might have given us the 30 percent survival values, and had we used still another dosage schedule we would have obtained a curve that would have given us the 60 percent effectiveness value, so that we must define what is the effectiveness of the schedule we are using. It is impossible ever to find a line above which we will get all of our recoveries and below which we will get all of our failures. There is going to be a variation depending on a thousand reasons. This is the first point that we have to understand: each one of these lines corresponds to a certain degree of effectiveness.

Now, because the normal tissues are not able to withstand unlimited amounts of radiation, we are going to be shy the degree of effectiveness that we would like to achieve, that is, a 100 percent recovery rate.

The other point that we have to remember is that the dosage line simply indicates two factors, the total dose and the total duration of the treatment, but dose will vary depending on the volume that has to be irradiated. In order to give validity to any such relationship you have to specify the volume distribution and the total volume, the quality of radiation employed, and several other factors. In other words, these graphs are not supposed to have universal validity. They are meaningful only for a specific set of conditions, and I think that we are losing sight of that fact in attempting to say that five-year observations are useless. We are measuring a certain effect, survival for five years, and we are trying to discover from the data what dosage brought about that effect under the circumstances that we stipulate.

I would disagree with Dr. Andrews concerning the absence of variation in effect with dose variations of plus or minus 20 percent. We were able to isolate a sample of cases that had fairly uniform treatment, and we showed with this sample that as uniformity in the material was brought about, there was a very definite increase in the proportion of recoveries as biologic dose increased, and vice versa. Of course, you have to have the same stage distribution. You have to have the same total volume and you have to have more or less the same schedule of treatment.

Unless we are very specific as to what these lines mean, we are going to be lost in conflicting opinion, and we are really not going to arrive at any better approach to the facts.

Dr. Kohn pointed out that it may be true that beyond 30 days a new factor comes into play that makes the dosage increment very much less as time goes on. Our data are not sufficient to be able to say which proposition is correct, his or ours. We tried to fit the simplest relation that would give us a reproduction of the data, and we tried to avoid using multiple terms because of this enormous biologic variation. We defined the dosage given by the equation as the median effective dose for the whole material. Then on reviewing the material by stages, we found that 60 percent of the defined dosage would give us 50 percent recovery in stage 1 cases, whereas in stage 3-B, that is, those who have bilateral involvement without any complications, the dosage has to be about 112 percent of the value given by the curve. We find that if we give too much radiation to the periphery of the pelvis the range of the dosage immediately changes. The mean effective dose goes up. The same thing is true if we have infection. On the other hand, if we are dealing with carcinoma of the stump the whole line moves downward. There is an infinite number of variables and we have to be as specific as possible in order to find the truth.

CORNFIELD: Dr. Friedman made one remark that I was going to address myself to later, but I might just as well do it now. The remark that interested me was the statement that if you take two groups of animals and treat one of them one way and one of them another way and then wait a long

period of time and observe the difference, a lot of things have happened in the meantime and consequently your results are invalid.

First, I would like to show you that I think there is one sense in which this is true but there is another sense in which I disagree with it. I think the sense in which it is true is that to the extent that there is a difference between these two forms of treatment, the more uncontrolled variation you permit to intervene between the treatments the more likely you are to blur this effect and not find it even though it is there. In that sense I would agree with the remark that something may exist and you may not find it because of this long interval. But the sense in which I disagree with it is that if you do find a difference between treatment groups and have randomized your animals between the groups, then as nearly as I can see it would be perfectly valid to say it was the treatment that was the cause of this difference, no matter how long the interval between treatment and observation of effect.

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2. RBE Versus Energy and LET

Victor P. Bond

I would like first to outline very briefly what I would like to talk about. RBE is a large subject. The discussion could take many directions. I would like first to define RBE, then I should like to deal with microorganisms very briefly to illustrate some of the principles and what we know about RBE, and then go on to mammalian values and discuss where the entire subject may possibly fit into radiotherapy, both at present and in the future.

As Dr. Kohn said, I have nothing very profound to add that you are going to take home and apply. Much of what I say is already known and published, but it may at least confirm some of the results that you have obtained clinically.

I am reminded of a book written by Douglas Dewar which he called, Difficulties With the Theory of Evolution. He then wrote a second one which he called, More Difficulties With the Theory of Evolution. From my conversations with participants here, this discussion may degenerate into a discussion of the difficulties with RBE.

Now briefly to define the term RBE. There are many ways to define it. As I want to use it now, it is the inverse ratio of the dose of a given radiation to produce a given degree of biological effect to the dose of a so-called standard radiation that will produce the same degree of effect. When I say dose here, I mean as the ICRU has recommended, tissue dose in r, or better yet, absorbed dose in rads. I do not mean air dose or some other unit. It is possible, of course, to use biological units of dose, for instance the skin erythema dose (SED). But I would like to define dose now as the physically measured dose. If anybody wishes to use other units of dosimetry he may do so, if he indicates clearly how and why.

I don't think LET requires very much defining here. It is linear energy transfer. It is equivalent, except for emphasis, to REL or rate of energy loss, and for our purposes means the same thing as specific ionization. LET, linear energy transfer, apparently is the term most acceptable to the physicists.

LET can be varied over a very wide range. As you know, the theoretical minimum for particles approaching the speed of light is about 0.2 kev/μ. This can be carried up to the order of 60,000 kev/μ. Of course, particles with the same velocity and equal charge will have the same LET in a given medium.

In a sense when we speak of LET with x- and gamma radiations we are dealing essentially with energy dependence. But there are other difficulties with energy dependence, so they are not precisely the same thing. We will get into that later.

Now to review briefly what is known about RBE. Speaking first of microorganisms, there is a great deal of data available. I will cite some specific examples, and particularly the data of Dr. Zirkle who has done excellent work on the subject. I think the reasons for using microorganisms for studying RBE are fairly apparent, but I might review these briefly. When we study RBE versus LET we like to have the LET remain constant throughout the volume of the organism being studied. There are a number of reasons this usually is not possible or is very difficult to obtain in a large organism. In the first place, if we start out with a beam with a relatively low LET, or range of LET, it is degraded in air, it is degraded as it goes through the specimen, and furthermore the LET varies along the path of the particles. If we use a beam with a high LET it has very poor penetrability. You cannot use alphas for studying RBE in mice under ordinary conditions. So to get the same LET, the diameter of the object to be studied must be small enough that the LET of the beam as it traverses the organism will remain reasonably constant. With x- or gamma radiation we have little difficulty with this problem.

Some of the best work on RBE that has been done is that of Zirkle in which either single track segment techniques with alphas have been used, or the beams from high energy accelerators have been used. These accelerated particles are usually deuterons, protons, or alpha particles, and such studies have been carried up to stripped carbon nuclei and beyond. There are other feasible methods of producing high energy or high-LET particles fairly uniformly distributed through a mass of tissue. These include the use of neutrons which give off protons in the tissue, and we can also give B^{10} or Li^6 to the organism or animal and then irradiate with thermal neutrons. The B^{10} or Li^6 essentially fission, and we get the release of heavy particles. These procedures have the advantage that the "carrier," the neutrons in this case, does have penetrability that is roughly comparable with x- or gamma radiation. But this situation obviously is not as "clean" as what we can get by direct irradiation of the organism with the particles or beam under study.

Now let's look at what has been demonstrated in microorganisms. There is, as I said, a very large literature. I will take just a few examples, and all I want to do here is to illustrate the principles. In these curves (Figure 1) which I have taken directly from Dr. Zirkle's recent review,[1] we have RBE versus LET. The LET has been varied over a range of from less than 1 kev/μ to 100 kev/μ. The point I wish to illustrate here is that the shape of this curve can vary greatly. In some instances the effect increases with increasing LET. In other cases the RBE decreases with increasing LET. In other cases it remains constant over a wide range of LET. In general I should say that to obtain a marked difference in effect it has been necessary to vary the LET by a factor of approximately five or more.

Figure 2 was taken from the data of Joseph Sayeg[2] when he was at the Donner Laboratory. Here the strong guiding hand of Dr. Zirkle was felt, I am sure. Here LET was carried beyond that used in the early experiments, and the same organism has been studied over a very wide range of LET. Here we are dealing with stripped carbon nuclei in the high-LET range. What I want to point out is that with changing LET, the RBE first remained constant, then went up, went through a maximum, and came down again. I believe this is undoubtedly a real phenomenon.

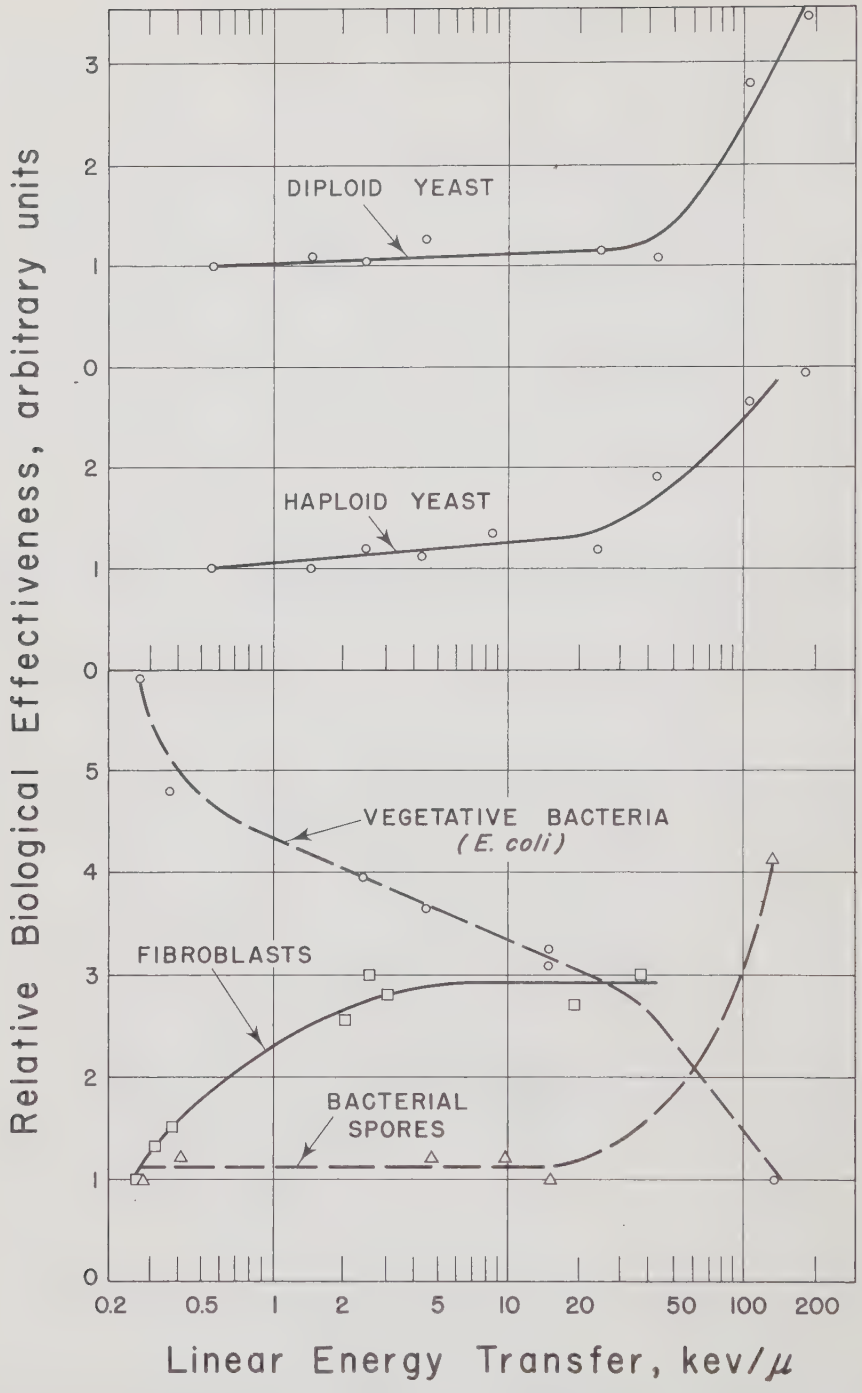

Figure 1. Variation of biological effectiveness with linear energy transfer (LET).

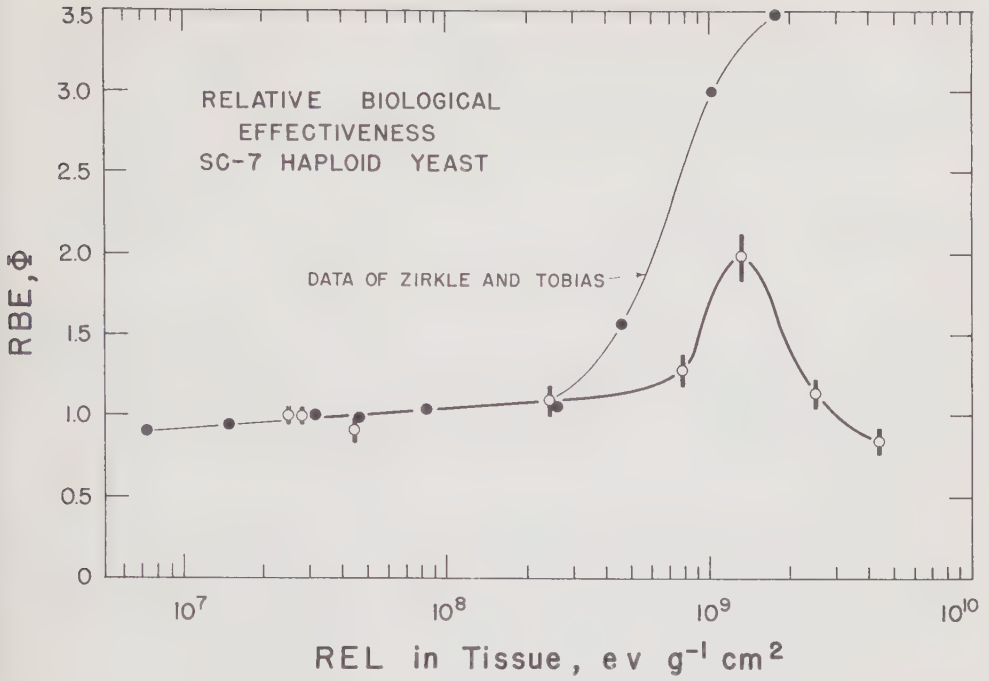

Figure 2. The relative biological effectiveness as a function of the rate of energy loss for various radiations.

What I want to do now is put the curves on these two figures together. I think it is not too difficult to see that any of the curves in Figure 1 can be fitted on a portion of the lower curve shown in Figure 2, depending upon where one is in the LET spectrum. In other words, the RBE either remains flat, goes up, or goes down as LET changes. Drs. Zirkle and Tobias developed a modified hit theory to account for this phenomenon. As a matter of fact, Dr. Zirkle predicted that this would occur, that RBE would come down again with increasing LET. I don't have time to go into the theory. I think the important point as far as we are concerned is that one can get almost any type of variation of RBE with LET depending upon the object irradiated, the endpoint used, and the interval of LET that is employed. There are not sufficient data to prove this, but theoretically, for most biological objects, if one could go through the entire gamut of LET, it would follow this curve.

EVANS: What is the biological object represented by the middle curve of Figure 1?

BOND: That is a curve for vegetative E. coli. The lowest curve represents bacterial spores--those of Bacillus mesentericus. Here two forms of similar organisms showed markedly different curves.

KOHN: This is only a detail. You don't really care particularly where that vegetative bacterial curve is.

BOND: I think that everybody will agree that RBE depends so much on

who did the dosimetry and on many other factors, that to place these curves exactly on the graph, vertically or horizontally, is extremely difficult. I think the important thing is the fact that if you put it all together it seems fairly definite that the RBE can remain flat, can go up, or can go through a maximum and come down again.

WOOD: There is an objection to the fibroblast curve because you cannot go to a LET of less than 0.2 kev/μ. If the curve were exactly as you have drawn it there, you could never get the plateau region.

BOND: That is a good point. Actually, the curve does not start at 0.2 kev/μ, but at approximately 0.3. Either the curve would have to rise rapidly over a very small interval of LET, or it is quite possible that some organisms or biological endpoints show a response only over a limited portion of the entire theoretical curve. It is also possible that the absolute value of kev/μ may be in error.

Among other difficulties with RBE determinations, the results depend upon other things that have been brought up--oxygen tension, pH, etc. One point which we don't have time to go into is that the higher the LET of the particles the less the effect of oxygen and other environmental factors. We won't go into Dr. Zirkle's theory to account for changes of RBE with LET. It is well written up in his chapter and we don't have time for it.

I should like to go into specific data now on the mammal. I think it is customary at this point to go even further with qualifying statements than in the case of microorganisms. Therefore, let me say that any results in the mammal must be interpreted with great caution, because of the difficulties of such work.[3,4] But I think we have to work with what we have. I have spoken of some of the added difficulties that we run into when we get to the mammal. Of course, dosimetry is one of the main ones, and RBE will change as the dosimetry is improved. I think that for general purposes this could be taken as a refinement and it will alter only very specific situations.

The RBE differs markedly with the endpoint used, and it differs for different endpoints within the same animal. For instance, if we take the RBE for spleen or thymus weight loss in the mouse, this may be different from that for mortality, and certainly it is different from that for cataract formation. There are other difficulties such as the so-called abscopal effects, which will be discussed in a minute. The RBE may also depend upon the time after radiation at which the reaction in a given tissue is measured. Dr. Stone can verify that the RBE for early effects may be quite different from that for late effects in the same tissue. Although the difficulties are myriad, I think that we still can make some sense out of such mammalian RBE data. It isn't quite as bad as it first appears.

These are some curves representing the variation of LD_{50} of the mouse and dog with energy (Figure 3). (The letters near the points denote the investigator.) I have used the energy here rather than LET because I think it is more familiar to most of us, and some changes that we observe here are not due to LET. As a matter of fact, I think many of the so-called effects of LET in the mammal are probably due to reasons other than LET.

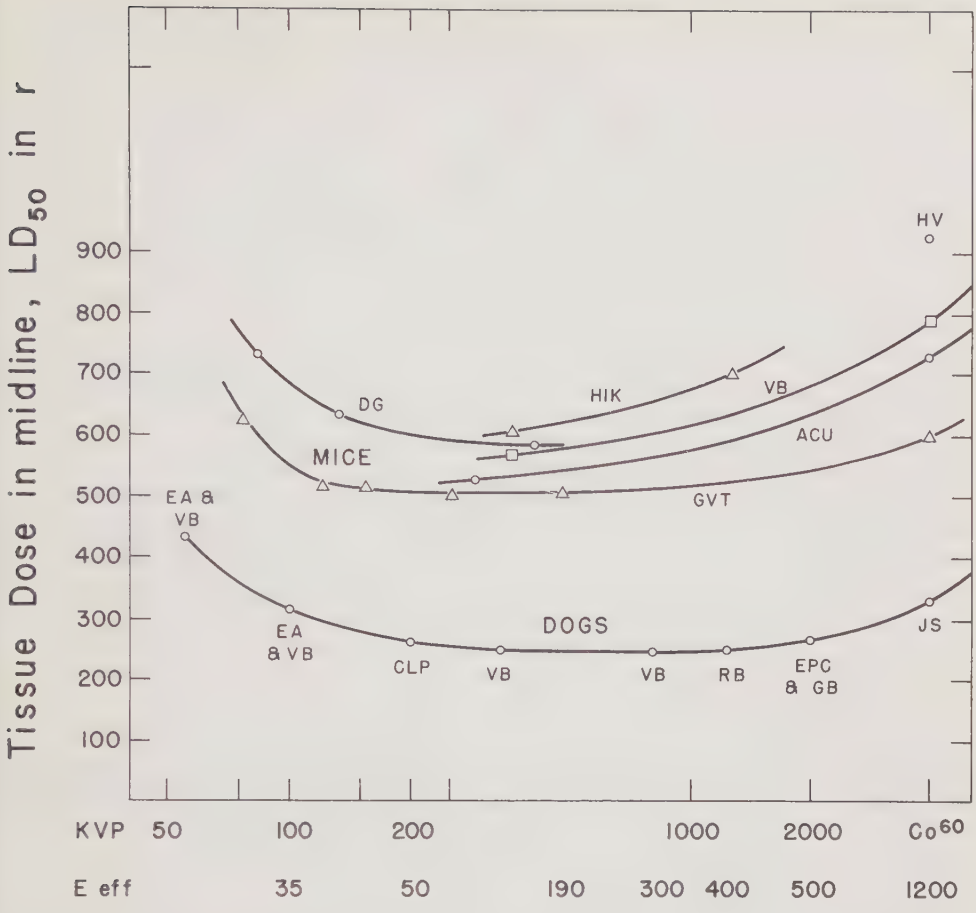

Figure 3. Curves representing the variation of LD_{50} of the mouse and dog with energy.

There are several things I should like to point out in this figure. First, the RBE seems to be flat over a fairly wide range, particularly in the dog, and it goes up as we get out in the region of Co^{60}. I am quite well aware that this may be on the basis of dosimetry and there will probably be some argument about this. But these are the data that are available now. Perhaps somebody will wish to comment on the degree to which the apparent change at the high energy end is due to dosimetry. Now I should like to point out that the curve, although it goes up at both ends, does so for different reasons. Its shape at the high energy end, as stated, may be related to LET or to dosimetry. The shape of the other end, however, represents a different phenomenon: degree of penetration.

I understand that in this meeting we are allowed a fair degree of speculation. So I should like to point out that the curve in dogs remains flatter over a wider range than the curve for mice. There are many data on mouse LD_{50} values in the literature. I happened to pick out the data of Dr. Kohn because

I think the dosimetry was done very carefully. He showed that the LD_{50} was definitely higher with 1,000 kv X-rays than with the lower energy X-rays. With the dogs, however, it seems to remain flat beyond this point. I wonder if the reason for that isn't that with the larger animals we have more degradation of energy in the tissue so that we are dealing with effectively a more uniform energy in the dogs, despite the KVP change, than we are with mice. I wonder as a result about the applicability of an RBE determined in mice to a situation in which a large tissue mass is irradiated, as in some radiotherapy problems in the human being. As I said, that is pure speculation. I cannot prove it but I think it should be looked into.

PATT: How similar are these dogs?

BOND: They were for the most part mongrel dogs. Many values were taken from the literature. The dose, you will note, is expressed in terms of tissue dose to the midcenter, not dose measured in air.

Now then we can deal briefly with the lower end of the curve (Figure 3). In dogs this is obviously a depth-dose phenomenon. The dose is in terms of midline tissue dose, not air dose. The effect may be due to decreased penetration through bone. I don't know with certainty, but at any rate the curve does go up very sharply in the dog.

QUASTLER: That is bilateral radiation?

BOND: This is bilateral radiation throughout. The midcenter tissue dose has no particular significance except that with bilateral or equivalent exposure techniques, and with energies of the order of 250 KVP or higher, it conveniently approximates an average dose received by all of the soft tissues.

KOHN: The dose on the skin must be enormous with low energy X-rays.

BOND: It is, and the animals lose their hair and develop erythema. They don't die with the same clinical picture, or at the same post-irradiation time, as with higher energies. We had some dogs that survived 60 to 90 days before they were sacrificed.

KOHN: Do you want discussion now or later? It seems to me that the use of that last point (the experimental determination is obviously unquestionable) in this constant depends upon how you wish to define the RBE. If you say that the experiment is designed to study the same kind of energy distribution then, of course, the point is precluded but becomes an important empirical fact.

BOND: The point that you are making, Dr. Kohn, is extremely important. The situation becomes much worse when one goes to unilateral irradiation, where the LD_{50} dose goes even higher. As you state, when the dose throughout the object in question is not essentially uniform, we cannot properly refer to an "RBE." What concerns me is, under conditions of nonuniform tissue dose distribution, how or where in the tissue does one express the "tissue dose." The ICRU does not recommend specifically on this point.

KOHN: Yes, they do--well, I think in part they do because they say that you are treating a tumor.

BOND: The point is that the ICRU has concerned itself principally with the problems of radiotherapists and not the problems of radiobiologists.

KOHN: I just wonder if you don't need two terms to describe the results of such experiments. Suppose we said that we were going to talk in terms of the effective doses of two kinds of radiation, the effective doses being defined in terms of endpoint, not in terms of the mechanism by which the endpoint is reached. Death is an endpoint. You can kill the dogs in any number of ways. We compare doses which we call effective because they reach the same endpoint. In the RBE experiment perhaps we must add the additional qualification that we must, as far as we can do it, kill the dogs in the same way. The mechanism must be the same as well as the endpoint.

BOND: This applies to mice also. When mice are exposed to neutrons versus X-rays, they apparently die of different mechanisms at similar 30-day lethality levels. This illustrates the fact that when we think we are dealing with LET in mammals, so many other things can come in that LET in many cases is submerged in the process.

JOHNS: In these studies you should not use the expression RBE but rather relative death point.

BOND: I agree that some other term should be used when the dose is not essentially uniform throughout the animals. We started out by saying that we would define RBE in terms of dose in tissue. However, the term RBE should not be used under conditions where a single value to represent the approximate dose received by all tissues cannot be given meaningfully.

I think these are all very important problems, and I don't know the answers to them. Apropos of what you said Dr. Kohn, if one takes the LD_{50} for 5-day deaths with X-rays versus neutrons, one will get one RBE value. If one takes the LD_{50} for 30-day deaths, one will get a quite different value.[4] This is just another manifestation of what I brought out here, that the RBE depends entirely upon which endpoint one is using, and where in the LET spectrum one is working.

KOHN: I should like to emphasize the importance of specifying the endpoint in detail when the definition of the RBE does not require that the mechanism of death (i.e., the mechanism of reaching the endpoint) is to be the same for both radiations. I don't want to give the impression that we should not know the mechanisms; you know that we understand very well what you mean.

BOND: What you are saying is one part of the much larger problem in RBE. It illustrates the fact that in our present state of knowledge there is no single RBE. It is still limited to the specific situation and how you define it. You have no assurance, even if you take the 5-day deaths with neutrons and X-rays, and thus presumably the same mechanism of death, the animals are really dying by the same mechanism.

KOHN: Perhaps this will bring up the need for a qualifying term here. There are some situations in which we feel that the mechanisms by which the endpoint is reached are rather closely related. I would say that is probably true for the X-ray range in which clinical radiation therapy is practiced. On the other hand, with neutrons I would just repeat what you have said. There are some parts of the spectrum, however, especially those parts that are of interest clinically, where this difficulty is not insurmountable.

KLIGERMAN: Do you think that you can use information gained from whole-body irradiation experiments in animals and expect it to be of help in the clinical problem of treating a limited tissue volume within a human patient, that tissue being a tumor? I don't think so.

BOND: There are two answers to this. I thought this meeting was to be give and take between radiobiologists and radiotherapists. We have discussed some of the problems of the radiobiologist with which I think the radiotherapist should be familiar. Also the radiotherapist may well contribute to the problems of the radiobiologist, particularly where dosimetry is concerned. I agree that LD_{50} studies in animals probably should not apply directly to radiotherapy. However, it seems to me that the problems encountered and answers obtained in large area or whole-body irradiation of animals should be fundamental to, and apply to the problems of radiotherapy. The LD_{50} merely represents a convenient endpoint.

KLIGERMAN: This is large-area irradiation but it is not whole-body exposure.

BOND: Radiotherapists are using whole-body irradiation in radiotherapy; Dr. Fletcher is. I agree with you that LD_{50} values on animals do not apply directly to radiotherapy, but I think many of the points brought up here are applicable.

I assume that radiotherapists do now conform with the recommendations of the ICRU and do use either tissue dose or absorbed dose in rads. This is not generally true of the radiobiologists, and they still, especially in this country, widely use air dose rather than tissue dose.

We have gone through some of our own data on LD_{50} values, and through the literature, and have taken the air dose values and converted them into absorbed dose (Table 1[5]). A different picture is obtained with absorbed dose than with air dose. We have listed small animals up through the rabbit and the monkey (Rhesus). We considered large animals as starting from the dog and going up to man. The LD_{50} value for man is highly questionable and is best omitted in this discussion. The absorbed dose is that delivered to the midcenter of the animal. The LD_{50} values seem to fall into two clear-cut categories when one converts to tissue dose. All small animals seem to fall into a high dose range; all large animals fall into a low dose range.

You remarked, Dr. Patt, that with a wide variety of "strains," shall we say, of dogs, there is a uniformity of LD_{50}. Here even with a variety of large animal species there is a uniformity of LD_{50}. In contrast, among the

Table I

Species	Radiation	LD_{50} (r)	LD_{50} (rads)
Mouse	250 kvp X-ray	500	638
Rat	200 kvp X-ray	640	796
Hamster	250 kvp X-ray	460	586
Guinea Pig	200 kvp X-ray	337	400
Rabbit	250 kvp X-ray	800	735
Monkey	250 kvp X-ray	550	522
Dog	250 kvp X-ray	304	250
Swine	1,000 kvp X-ray	510	247
Sheep	Nb^{95}-Zr^{95} gamma	524	205
Goat	200 kvp X-ray	350	237
Burro	Ta^{182} gamma	651	256
Man	Fallout gamma	350 (?)	300 (?)

small animals there is considerable variation with strain and species. I don't know why this difference should exist, but I point it out for two reasons. First, because I think it is interesting, and second, it shows how one can be misguided or misled by the use of air dose rather than tissue or absorbed dose.

JOHNS: Isn't that the dose in roentgens at the midline?

BOND: The first column is the air dose, measured at a point corresponding to the proximal skin surface. This is the dose measured in air as the investigator usually measured and reported it, and in terms of which RBE is frequently reported. The absorbed dose, of course, is what should be used for the RBE.

STONE: I should like to rise to the defense of those using the air dose, not that it should be used but in a great many instances you may not know anything except the air dose. If a person is working in a field contaminated with gamma radiation, for example, you don't know which side he is exposing and you don't know whether he is getting it on both sides or in the center.

BOND: I certainly agree with you, Dr. Stone. My only point is that when your conditions can be controlled, then I see no excuse for the use of the free-air dose. This point is violently argued by some radiobiologists. I don't see their point, frankly, but they refuse to use tissue or absorbed dose under conditions where they could just as well do so.

So much for the lower end of the LET spectrum, essentially the x- and gamma radiation. Now let's get to the higher end of the LET spectrum. Again there is a great deal of data, early and recent, on the variation of RBE with LET. (Figure 4) This figure was taken from a recent report of the Los Alamos group.[6] I suppose the statisticians might be shocked at fitting such a curve as this, but again you work with what you have.

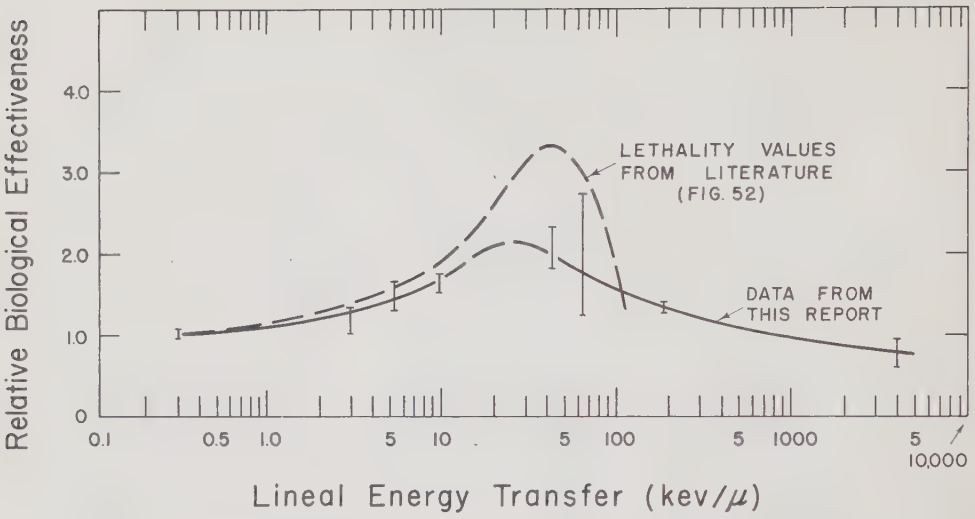

Figure 4. Relation of RBE to LET for mammalian systems.

The upper curve represents data from the literature and involve some of the individuals here. Some of these points are our own, and some of them are Dr. Evans'. To identify roughly the radiations represented by the kev/μ values shown on the graph, we may recall that X- and gamma rays are in the range of a fraction of one, to a few, kev/μ. Most recoil protons from neutron interaction with hydrogen lie in the range of roughly 10 to 100 or more; the "average" LET for a fast fission neutron spectrum is approximately 50 kev/μ. Radon alpha particles have a LET of 110 kev/μ. Fission fragments represent several thousand kev/μ.

The lower curve represents data from the Los Alamos group, and it has the virtue that at least the work was done in the same laboratory. The same group did these, although not necessarily the same individuals. Apparently mammals follow the trend of going up slowly in the low-LET region, and then rise in the neutron range, coming down again in the alpha range and still further down when one gets to alpha particles, to recoil particles, and to heavy particles from n-alpha reaction with B^{10} (about 200 kev/μ). The point at 4,000 kev/μ, I believe, represents the RBE for fission product fragments. So it would look like the mammal curve follows roughly that obtained with yeast.

ZIRKLE: What is that point with the very small probable error? Is that alpha?

BOND: That would be about 200 kev/μ. It represents the recoil particles from the B^{10} (n, γ) Li^7 reaction.

ZIRKLE: In the upper curve, does the very last point at the right represent the boron disintegration?

BOND: Yes, I believe so. The LET actually is a little higher than that shown.

ZIRKLE: Lorenz with his alphas got something very close to that.

BOND: Except that he didn't have uniform distribution of dose. We don't claim uniform distribution either, but ours is more uniform. There is a large concentration in the kidney, for instance, with Lorenz's data. In ours the distribution is roughly similar to that of body water, and fairly uniform.

ZIRKLE: It is very interesting that the Lorenz data falls on the curve despite that.

KOHN: The alpha particles are given by injection?

BOND: Lorenz did, by injecting radon. We gave B^{10} by injection, and then irradiated with thermal neutrons to obtain the B^{10} (n, γ) Li^{7} reaction.[7]

Now to summarize briefly where all of this might fit into radiotherapy. I think in the X-ray, gamma-ray range this has been covered pretty well. It seems a question of arbitrarily deciding which RBE value to choose in the high energy range. I have no recommendation.

As for the possible uses of high-LET particles in radiotherapy, I think there are several possibilities, and I should like to point out a few considerations in this regard. One is that the RBE's as found now are generally lower than previously reported. For acute effects, the RBE for heavy particles is down around the order of 1-1/2 to 2. The values recently determined are not the 20 or so that have been reported.

Second, most RBE values reported to date are for short-term effects, and they are not necessarily applicable to long-term effects. Again they represent sensitivity, not curability, but perhaps someone may want to take advantage of this factor of 2 or 3. There are several ways of doing this. One that has been talked about is taking advantage of the tail of the so-called Bragg curve for high energy particles such as protons or deuterons. This has the advantage that at the tail of the curve not only is the dose higher but the RBE is higher, presumably because the energy is lower. This possibility was discussed at Oberlin and I think pretty well squelched. In the first place, the tail of the Bragg curve is very narrow, and in the second place one would have a great deal of difficulty localizing this to the lesion in question because of anatomical considerations.

It is possible to use a high-LET beam, such as neutrons, as we would use an X-ray or a gamma-ray beam. Because of the variability in the RBE values obtained as a function of endpoint, however, before one could conclude that there is any probability of a differential effect, one would have to show, under the actual conditions to be employed, that there is a higher RBE for the specific tumor involved than for the adjacent normal tissue.

KAPLAN: I think we will call for a little discussion on this subject.

CANTRIL: To answer partially the question whether this fits into radiotherapy, as one goes about the various clinics where high voltage energy radiation is being used, one finds that some clinics are putting an RBE factor into their tumor dose while others are not. I think this in itself is of some importance. As to whether such a concept is or is not valid in view of what we know about RBE from animal data, and considering the difficulties of measuring RBE with a variety of specific endpoints, with problems in comparable dosimetry, acute versus long-time effects, variations between animal specifies, etc., I would find it difficult to extrapolate this to human RBE for normal tissues or tumor. The variance in biologic effectiveness between 250 kv and gamma rays of radium or 2,000 kv X-rays is small and other factors which enter into one's therapy, namely, protraction fractionation, tumor volume, the type of tumor and its biologic response, the general condition of the patient, etc., are factors which may have more importance than any unknown variable in RBE.

BOND: Don't get me wrong. I am not advocating that.

CANTRIL: No, I know you are not. Dr. Kligerman brought this up. For these reasons we do not enter an RBE factor for tumor dose with X-rays in the range of 2,000 kv. I know that there are some who do.

KAPLAN: I want to correct that. You do enter an RBE factor. You just assume that the RBE is 1.0, whereas the people who are being described as putting a factor in are assuming that it is other than 1.0. You have no better basis for assuming that it is 1.0 than they have for assuming that it is different from 1.0. That is a more precise definition of what is being done.

CHAMBERLAIN: May I protest? If you express your dosage in rads you are not assuming any RBE factor at all, and it is only when you try to express it in rems that you are. You may think in rems. I think that we should think in terms of trying to arrive at a rem dosage also. If you put in rads and you calculate for rads, you do not put in a correction for RBE at all.

KAPLAN: You are applying an RBE to your choice of total dose levels.

CHAMBERLAIN: I am not sure that people who change dose levels have RBE alone in mind, or whether they are also considering the coefficient of absorption.

CANTRIL: I think they have in mind an RBE factor and they hence may add X rads to their dose in view of such assumption.

NICKSON: There are in the literature statements by groups that they are making the assumption that the RBE is less than 1.0 and, therefore, their total dose interrelationships are adjusted by this factor. In partial answer to Dr. Chamberlain's statement that we don't know whether the RBE is different, strictly speaking I think this is true. On the other hand, the systems that have been tested when the energy is expressed in rads do not depart from 1.0 within the limits of the experiment, and I think it is legitimate also to cite the clinical observations. Here it seems to me that within the limits of variation,

there is no detectible deviation of RBE from 1.0 in man at the dosages which we are using in patients.

FRIEDMAN: That is a personal opinion.

NICKSON: It is a personal opinion.

CANTRIL: I would correct that to a personal observation.

NICKSON: It is of some interest that one group, using a betatron, have in the past two years said that in view of their clinical experience they have stopped using an RBE factor of 0.7 in selecting total dose levels and are now assuming an RBE of 1.0.

BOND: You have facilities apparently for calibrating directly in terms of rads, using calorimetry, I believe. How about the individuals who don't have equal facilities and have to rely on more commonplace dosimetric devices? I assume that what you are saying here is that your dosimetry is different from or improved over, that of other people and that this may account for your observed RBE of 1.0 where others have reported values other than 1.0.

NICKSON: I don't know what Dr. Kohn is going to say, but when we converted dosage from roentgens to rads (with one exception, and we think we have a biological explanation for that) the apparent RBE differentials drifted back to 1.0 within the limits of experimental observation. This includes a reasonably broad spectrum of biological indicators.

KOHN: The last sentence clarifies it all for me. I didn't realize that you were speaking specifically of the betatron. I know of no certain evidence from the laboratory point of view that would contradict that statement. I only meant to imply that perhaps there are some data in the range of energy between 200 kv X-rays and the betatron which indicate that there may be some small differences of the order of 10 or 15 percent.

NICKSON: I agree wholeheartedly with Dr. Bond's comment that when you talk about RBE you have to define your time and place. You have to be very careful about having it spread over to other endpoints or other energies.

ROBBINS: Could we ask Dr. Fletcher to comment on what he observed with the base of the tongue lesions?

FLETCHER: Of course, we are interested in the subject and we have published some of our observations.[8] In the oral cavity, primarily the oropharynx, we selected one area of the mucous membrane which we could observe and which would always be the same, that is to say, the mucous membrane of the soft palate. We treated a number of cases on a comparative basis, trying to deliver essentially the same dosage to that part of the soft palate. We have not only recorded cumulative doses but actually plotted volume distribution.

Then we started a schedule of giving 6,000 r in four weeks, five weeks,

or six weeks. The choice actually depends on the size of the lesion. If we have a small lesion we give it in four weeks. We distinguish different degrees of mucositis in order of increasing severity as: red +, red ++, studded and confluent. We have found that there is a curve of this sort (Figure 5).[8] At about the end of the second post-treatment week you see a confluent reaction which continues for about two weeks or so and then regresses.

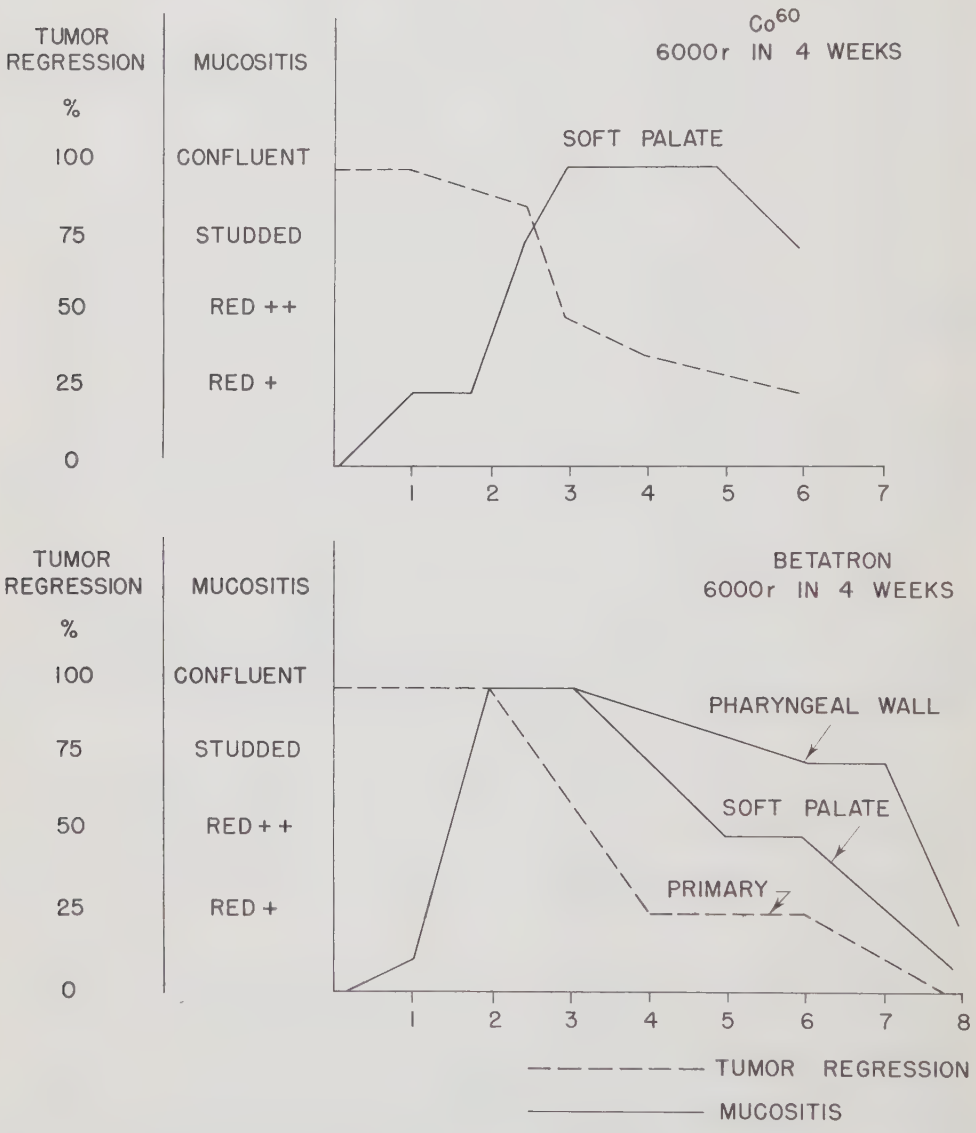

Figure 5. Graphs of mucositis in two cases of tumors of the tonsillar fossa, one treated with the Co^{60} and the other with the betatron. It is obvious that the time of development of the confluent fibrinous mucositis and the peak are the same. There may be a slight difference in the healing slope.

You can duplicate those curves with a remarkable degree of accuracy. There is always a confluent mucositis at that dose level at about that time. If you give the same dose with the betatron or the Co^{60} unit, you get exactly the same clinical response in terms of mucositis. As far as one can see, I don't think there is any perceptible difference. So 6,000 r in four weeks with either Co^{60} or the betatron gives the same mucous membrane reaction.

With 5,000 r you get a curve that slopes and does not quite hit the confluent stage. It may at times scatter in individual curves but it is mostly a studded membrane reaction.

Now, interestingly enough, if for instance you treat a case of carcinoma of the tonsil on the betatron, using parallel opposing fields and a 2 to 1 differential field dose ratio, you will see a graded mucous membrane response from one side to the other. You will get an isodose distribution maximal at the involved tonsil, where the isodose value is 90 percent; then the isodose at the uvula will be about 80 or 85 percent, and about 75 percent at the opposite tonsil. That is to say, roughly a gradient of about 15 percent, with corresponding gradation of mucositis from red at the far tonsil to confluent at the near tonsil. Thus the biological response reflected in those three degrees of mucositis is sensitive enough in this particular experiment to reveal a dose difference of 15 percent. If you transfer that dose into rads, the rads have a coefficient of 0.97, and in roentgens it is 0.92, I guess.

JOHNS: Ninety-three.

FLETCHER: It is not quite as close as with the cobalt. With the betatron, 6,000 r are pretty close to 5,700 or 5,800 rads and with the cobalt, say 5,300 rads. I don't remember these figures. Would you say that the betatron rad is somewhat more effective than the Co^{60} rad? I don't know. That is what one seems to get. I am not taking any credit or blame for it.

When you try to do the same comparison with 250 kv, you have to take the same biological endpoint, of course. I am going to talk like the statisticians. I am not talking any more about RBE reaction. I am talking about endpoints. If we take the same endpoint, the reaction of mucous membrane on the soft palate, we must always keep it constant, because the pharyngeal wall does not have the same severity of reaction. The base of the tongue and the dorsum of the tongue, for instance, are much more resistant and you have to give much more.

When you try to do this experiment, you begin to get into trouble. I could not give doses of 6,000 r at 250 kv to the tonsil or the base of the tongue. For that matter I would feel unhappy about taking a patient with carcinoma of the base of the tongue or the tonsil and treating with 250 kv X-rays. I think that I would be doing him an injustice. You can treat carcinoma of the nasopharynx quite well with 250 kv, however, so we took a number of nasopharynx carcinoma cases and tried to see what we could do. You can use two to four fields. We used two laterally and two in front, with 250 kv, going to a midline tumor dose of 6,000 r in six weeks. Of course, the soft palate and the uvula are in the beam and, therefore, you can observe them. Then we tried to measure

this but there is a fair amount of variation. In front the beam traverses the cavity of the antrum and your dosimetry is off by 15 or 20 percent. What struck me is that there is very little variation from patient to patient. Those curves are strikingly the same. Last year I had about 20 patients in each of these groups and now I think there are about 50. The responses are constant. You can predict. As a matter of fact, if the patient is going to go home and is due to come back in 12, 14, or 17 days, we tell him that he is going to be most unhappy. This particular endpoint is really very accurate, and a difference of only 10 to 15 percent in dosage, as I said, will alter the response from a very mild mucositis to a full-fledged confluent membrane. So, if your dosimetry at 250 kv is erroneous by 10, 15, or 20 percent you might as well give up. We tried to apply corrections for bone and air absorption. I would say this is not quite the same geometry any more.

Actually I got the idea from Dr. Friedman many years ago to plot the degree of mucositis. He does it extensively and he also plots the tumor regression. Mucositis and tumor regression are the same either with Co^{60} or with the betatron.

If I may take one more minute to show the difficulty of the problem, suppose we try to do the same thing for tumors within the pelvis. For a number of years, we treated our more extensive cervical cancers, including some early cases that were not suitable for radium alone, giving a 2,000 r dose to the midpelvis in four weeks and this was tolerated very nicely. Dr. Bloedorn thought it was a very long time and probably not an effective dose, and he was right. He said "Suppose we do that a little faster?" So we gave 2,000 r in two weeks. We did not change the geometry, but we had to give that up because the patients could not tolerate it. We moved 2,000 r in three weeks. This is our present schedule when we use 250 kv X-rays. That is tolerated but is just about at the limit. An occasional patient will have a little difficulty with diarrhea. That is an endpoint, you see. You get diarrhea as an endpoint. On the betatron our schedule is 1,000 r per week, and we give 6,000 r in six weeks, or 4,000 r in four weeks. At this dose rate, just occasionally a patient will have some mild diarrhea. Two thousand r with 250 kv X-rays in three weeks seems to be the equivalent of 2,000 r in two weeks with the betatron. Now how are you going to figure that dose? That means a ratio of 0.66. You do not get the same result in the pelvis as you get in the oropharynx.

STONE: On your betatron treatment if you went on for three weeks you would still have the same reaction from 3,000 r in three weeks. So your RBE would change to one by then.

FLETCHER: No, since we gave 3,000 r in three weeks with the betatron and we gave only 2,000 r in three weeks with 250 kv.

CURTIS: Several years ago we started to do a large series of RBE measurements on a wide variety of different biological materials, thinking that the RBE was a fixed cellular constant and therefore represented a basic characteristic of the cell or tissue in question. However, we soon found that for any given material one can cause the RBE to change markedly by treating the

material in various ways or by performing the irradiations when the material is in different conditions. This means that RBE is not a profound cellular or tissue characteristic but is much more a characteristic of the treatment which the tissue receives. For example, one can vary the moisture content in dormant seeds and change the RBE by as much as a factor of 50.

BOND: You can affect the RBE after the treatment? This is an important point.

CURTIS: You can do both. That is, the treatment of the material both during and subsequent to irradiation will modify the RBE over wide range limits.

BOND: But the wide changes in RBE you are quoting result from differential handling of the material during irradiation?

CURTIS: These measurements have led us to believe there is nothing particularly profound about RBE as such from a theoretical point of view. The important consideration is the way in which the radiosensitivity of the cells can change with various treatments both for neutrons and for X-rays. Differences in sensitivity, which is what the RBE measures, may give a very misleading picture of exactly what is going on.

From a practical point of view there is no question about the usefulness of the RBE concept. In health physics it is necessary to lay down certain maximum allowable dosages and here one must necessarily decide on a value for RBE and set the tolerance accordingly.

For the topic under discussion here, namely, the treatment of tumors, it seems to me there are some ideas from this work which will have definite application. Two things come to mind in thinking about applying these ideas to tumor therapy. They are based on the findings that X-ray sensitivity varies enormously with water content, oxygen content, the state of the metabolism, etc., whereas neutron sensitivity in general remains very insensitive to such changes.

The first of these possibilities is the guess that with neutron therapy, the radiosensitivity of tumors would be relatively invariant so if neutron dosage was regulated from a physical point of view then it would also be well regulated from a biological point of view and there would be a lot less guesswork with respect to the appropriate dose to use.

The second possibility is that all types of tumors would be equally radiosensitive to neutron therapy whereas we now know that their sensitivity to X-rays varies greatly. Thus, the possibility exists that many tumors which are now considered to be unsuitable for radiation therapy because of their extremely low radiosensitivity might be sensitive enough with neutron therapy to make it feasible to treat them with neutrons. The possibility exists that one of the reasons that some tumors are very insensitive to X-rays is that the oxygen tension within the tumor itself may be extremely low and this may be the cause of the insensitivity. If this is true, then it probably would not

hold for neutron therapy since in all systems so far studied the radiosensitivity to neutrons is independent of the oxygen tension at the time of the irradiation.

For these reasons I feel it would be quite profitable to reopen the entire question of neutron therapy of cancer, not with the idea that it will be a panacea but rather feeling that there are almost certainly some types of tumors which can better be treated with neutrons than with X-rays and perhaps some types of tumors which can be effectively treated with neutrons for which X-ray therapy is now contraindicated.

BOND: I see what Dr. Curtis is driving at, but I could adopt precisely the opposite viewpoint in regard to the theoretical importance of RBE. I could say that RBE is not of very much importance in health physics because of the long chain of possible errors that may enter from the reading of an instrument to interpretation of potential biological effect. One might leave his film badge on when going to the dentist, or forget to wear it regularly at work. In the face of the many potential human errors, RBE could be considered a rather minor link in the chain. When you say that RBE is not so fundamental I don't agree, because I think that when you get variations in RBE like those observed, this serves as a very useful tool with which one can study fundamental radiobiological action. Then when one is able to vary the environment and thus further modify RBE, this gives one an additional tool with which to study fundamental radiobiological action. I would add that until a good deal of very fundamental work is done on RBE, varying the endpoint and LET over a wide range and utilizing a wide variety of conditions--until we understand why the observed curves are as they are, we will probably not get to the point where RBE will be of great significance in radiotherapy.

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3. Possible Role of Abscopal Effects (Lorenz and Hollcroft); Status of Sensitizers; Status of Combined Radiation and Chemotherapy

Richard H. Chamberlain

I haven't any idea why Dr. Kaplan asked me to do this, because as I looked around, I found that almost everyone else in the room really knows something about one of these three factors, either abscopal effects, sensitizers, or combined radiation and chemotherapy. I was forced to do a little reading of the literature.

I do perhaps have one qualification in that I studied classical languages enough to give you a definition of what abscopal means. This is freely translated from Greek. It means target in another position, and so the concept is that you don't aim your radiation at the target but somewhere else.

So far as I can tell, the reason Mole[1] used this term is because the term "indirect" was already preempted at the cellular level and presumably he meant abscopal not to apply to intracellular phenomena but to some more microscopic type of biologic effect.

Clinically there are reports on such remote effects going back, apparently, to as early as 1906. Radiotherapists were interested in differences in tumor response, depending on whether they included other volumes of the body in the radiation field or not, and thought that there was some such influence to be observed. If one treats the ovaries with radiation and thus influences breast carcinoma somewhere else in the body, this is an abscopal effect of a type. In this instance, you know that the mechanism by which this is mediated is a hormonal effect. If you treat a lymphoma involving a regional mass of nodes and, as some people have thought, get an effect on nodes elsewhere in the same patient, which is doubtful, you don't know the mechanism. You might consider that possible evidence of another type of abscopal effect.

Furthermore, I don't understand what is meant by "volume," because some clinicians feel that perhaps there is a difference in treating keloids as to whether you confine the zone of irradiation sharply to the keloid or whether you include a considerable margin of apparently normal tissue about it. This is local abscopal effect, I presume, if there is really such a difference. Perhaps some of the experiments Storaasli and Friedell have done with combined radioisotopes may have some abscopal connotation.

To go back to what I could find in the radiobiological literature, I think the original contributions in this field were the Lorenz and Hollcroft experiments[2-4] in which the tumor response varied with the amount of whole-body irradiation given. The amount of radiation given locally to experimental animal tumors was supplemented with a variable amount of whole-body irradiation.

A recent paper[5] is that of Dr. Raventos, who is now in our department. He exteriorized the spleen and compared the response treating the whole animal and spleen versus treating the spleen alone. Incidentally, he also did some work on treating the animal alone and protecting the spleen, and he drew some conclusions of which he was not quite sure. More recently, Mendelsohn has taken his data and derived another explanation for them which is not abscopal at all in my understanding of the word. But the effect that Raventos found could be explained on the basis of a migration of lymphocytes into the spleen from the body.

Next we may go on to such things as the difference between in vivo and in vitro effects. With endpoints such as the pyknosis of lymphocytes, the amount of radiation required in vivo seems to be different from what is in in vitro. Mendelsohn is also trying to relate X-ray effect to stress reaction. He has treated adrenalectomized animals and, of course, has referred back to Patt's work on hypophysectomized animals[6] and the lack of difference in response. I think other work has been done on the role of the adrenals, too.

Thus, there may be some evidence for there being a difference in the response of local areas, depending on what is done elsewhere in the body, due to mechanisms that are poorly understood. There are the hormonal situations in which, at least for the ovaries and the testes, there seem to be distant effects of radiation. I don't know fully the work of Hardin Jones on the changes in DNA in distant lymph nodes on radiation of local ones. I am unable to derive any clear-cut idea as to how we can use this type of phenomenon clinically.

BOND: I should like to make a comment and to ask a question. Leblond and Segal[7] have reported an abscopal effect on the thymus and other lymphoid tissue. We did some work along this line and irradiated various portions of the animal with a high energy deuteron beam so that there was essentially no scatter. We could find abscopal effects in the spleen, thymus, or adrenals, as measured by their decrease in weight, only when the animals showed signs of stress. It is difficult to define stress, but the animals looked sick and lost weight before these changes occurred. If we irradiated, say, the hindquarters and sickness did not occur, we did not get the abscopal effect. I should like to ask if in clinical radiotherapy such a thing is observed. I should also like to ask if there are clear-cut evidences of abscopal effect in other than lymphoid tissues.

PATT: Did you say that you did not get an abscopal effect?

BOND: We did get it but only when the animal looked ill.

PATT: What is your interpretation of "abscopal"?

BOND: It means an effect distant from the site of irradiation.

PATT: There are many effects that can be interpreted as departures from a purely local action. The term abscopal was introduced by Mole to obviate a semantic difficulty, as Dr. Chamberlain has pointed out. In the

early experiments by Leblond and Segal, irradiation of the chest resulted in involution of the thymus and some involution of lymph nodes elsewhere. In adrenalectomized animals involution was restricted to the thymus. There are other abscopal effects that are not related to adrenal intervention, for example in Jolles' experiments on skin.

BOND: That is a very close effect.

PATT: It is still abscopal. Dr. Zirkle has suggested calling such effects micro-abscopal.

CHAMBERLAIN: And even intracellular abscopal effects, possibly.

PATT: No, it may not be within the cell that is irradiated. You can think of this on a molecular basis. The abscopal effect is one which is removed in time and space from the initial event of energy absorption. If you look at it that way, I don't see any difficulty in recognizing that there are abscopal effects and that these are undoubtedly of importance.

BOND: Would you name a few other than the response of the spleen and thymus, which presumably operates through a Selye stress reaction.

PATT: One need not know the mechanism.

BOND: All right, name a few abscopal effects.

PATT: Pituitary tumors resulting from thyroid irradiation; the interplay between pituitary and ovaries which is presumably necessary for some ovarian tumors after irradiation.

BOND: Let's come back to my original comment. I quoted Leblond and Segal, incidentally. We did dosimetry under their identical conditions and observed so much scatter beneath the shield that this could have been a direct effect on the spleen and thymus and not an indirect effect. The comment I made was that we have seen the same thing as Leblond and Segal but we have seen it only when we made the animal sick. The question I am asking is, do you see abscopal effects which operate through quite a different mechanism? Is something released at the site of radiation that produces an effect some place else under conditions in which the animal is not losing weight and is not sick?

PATT: You are raising another point. Are toxic materials released?

BOND: We are discussing a basic fact: are there clear-cut abscopal effects when the animal is not sick?

KAPLAN: I think we should remember the work of Hevesy,[8] later confirmed by Barbara Holmes,[9] though disputed by others.[10-13] They were measuring the effect on nucleic acid P^{32} uptake in transplanted mouse tumors and found a depression not only in the tumor that was irradiated, but in another tumor in the same mouse that had not been irradiated.

BOND: You said disputed by others.

QUASTLER: Disputed by Barbara Holmes. She says that much of the effect is due just to anesthetizing and tying the animal down at the time of irradiation.

KAPLAN: She confirmed Hevesy's work. Whether she explains the effect in other terms I do not know. What they did not do is remove the adrenals and see whether they still got a remote effect. I have always had the feeling that this is probably a stress reaction. The release of cortisone is enough to inhibit growth and protein synthesis and presumably also to inhibit incorporation of P^{32} into nucleic acid.

BOND: This is my question again: are there abscopal effects that are not clearly due to this "stress" mechanism?

KAPLAN: The only effect I know of that is pertinent for radiotherapists is the one that Lorenz and Hollcroft observed, which seems a different kind of response entirely. I don't know whether it is really proper to refer to it as an abscopal effect, but in essence, they found a greater effect on the regression and control of a locally growing transplanted mouse tumor (I confess this was a lymphosarcoma) when they shielded the tumor and gave supplementary doses of whole-body irradiation.

BOND: So it was a lymphoid tissue.

KAPLAN: Yes, but the effects they observed were pretty striking for adrenal effects.

CHAMBERLAIN: And they were dose-dependent.

PATT: Dr. Chamberlain mentioned, I believe, the experiments by Lick, Kirschbaum, and Mixer[14] on ovarian tumors. In the broad sense, aren't these examples of abscopal effects, that is, of the interplay between irradiated and nonirradiated areas?

BOND: Yes, but you still have not answered my question. In what state is the animal when you observe these so-called abscopal effects?

PATT: In these experiments, development of ovarian tumors required irradiation of both ovaries. If only one ovary was irradiated, there were no tumors. As I recall, there was no obvious stress reaction in their animals.

We may not be smart enough to detect early abscopal effects or departures from local action, but it would be remarkable if there were no interactions between at least some components in a highly integrated system.

FLETCHER: I thought I heard you talking about an experiment with another abscopal explanation. What is the abscopal explanation?

CHAMBERLAIN: The only thing I said is that this term has been used for things that we don't understand.

QUASTLER: In therapy one commonly sees profound lymphopenia and less profound granulacytopenia following heavy treatment of lesions anywhere in the body--

BOND: What endpoint?

QUASTLER: Lymphopenia.

BOND: That is clear cut? There is no question?

NICKSON: It is due to the adrenals.

BOND: I think so.

KAPLAN: In patients you see this without loss of weight. You see it without obvious evidence of "stress." There we may well be dealing with a bona fide abscopal effect. It is a clinical observation which really deserves further study.

NICKSON: There is a possible way of checking on this, because there are now a fair number of adrenalectomized patients who come for treatment. If the same response is seen there, you have a reasonably good basis for considering this a nonstress type of abscopal effect.

CHAMBERLAIN: I purposely omitted radiation sickness from this discussion because I am of the opinion that its clinical evaluation is almost impossible.

Under the heading of sensitizers, the only thing that I know of is the porphyrin work. Since Dr. Vermund is here, it seems to me that if anyone is going to say whether this holds any promise or has any real interest, it ought to be Dr. Vermund rather than myself.

VERMUND: The work done at the University of Minnesota was initiated by Samuel Schwartz. The observations of Figge, at the University of Maryland, were really the fundamental ones.[15] He observed that certain transplanted tumors seemed to concentrate certain porphyrins which he thought were hematoporphyrins. This was manifested by an increased fluorescence in these tumors as compared with the normal tissues around them.

Then Dr. Absolon, Dr. Schwartz and I carried out some experiments in which we irradiated transplanted carcinomas in mice, following injection of what we thought was hematoporphyrin.[16] It later turned out to be a mixture of a number of different porphyrins. The chemical problem of separating and identifying these different porphyrins is very difficult. There are still many problems which have not been solved. Actually there might be porphyrins other than the hematoporphyrins that are responsible for this differential uptake in tumors.

Briefly, what we observed is that there seems to be some potentiation of the radiation effects on the tumors in connection with the use of the porphyrins.

This has now been carried out on a rather wide scale by Dr. Schwartz, working very closely with the statisticians, and there can be no doubt that you can obtain a potentiation if you inject the animals with a low dose of prophyrins prior to the irradiation. We obtained equivocal evidence in our early experiments, probably because we gave excessive doses of porphyrins, and were very much in doubt about the conclusions initially. We have become convinced since we have seen the most recent data that Dr. Schwartz has collected, using many hundreds of mice injected with small doses of porphyrins, very careful randomization, double blind experiments, and so on.

When it comes to work on patients we don't know for sure what the effects are. We had to base our opinion upon clinical observation, which is very difficult. The patients we selected for treatment had very far advanced tumors which were considered to be radioresistant, or tumors which had had radiation before and had then developed local recurrence. It was the impression in some cases, particularly in the squamous cell carcinomas of the oral cavity, that there might be some initial increase in the rate of regression of the tumor, but certainly we were not able to prolong the lives of these patients to any extent and we gave up the whole clinical experiment.

Dr. Schwartz has now prepared a new porphyrin compound which is a copper complex. It does not fluoresce but still seems to potentiate radiation effects in transplanted tumors in mice.

STONE: May I ask the meaning of your word "potentiate"?

VERMUND: It increases the rate of regression of tumors as determined by measurements.

STONE: With the same amount of radiation?

VERMUND: That is right.

NICKSON: Is that the only endpoint?

VERMUND: No, we have also used the endpoint of survival of the animals following transplantation of the tumors. There is an increased survival of these animals that have had porphyrin treatment.

NICKSON: What happens if you use regression as an endpoint and not decreased rate of growth?

VERMUND: The same thing.

NICKSON: What is the magnitude of the effect?

VERMUND: I cannot give you the figures on this without referring to the actual data.

NICKSON: Is it a factor of 2, a factor of 1-1/2, or a factor of 1?

CHAMBERLAIN: I have this preliminary report here if you want to read it.

NICKSON: I should like to see it in the record, because the statement that there is such an effect has been made and it would be nice to have a statement of the magnitude of the effect.

VERMUND: The effect of porphyrins with regard to total-body irradiation is going to be reported during the meeting of the Radiation Research Society in Rochester. I think I should like to postpone the discussion of it until then. If you use large doses of hematoporphyrin prior to total-body irradiation you get a protective effect, just like you get, say, with cysteinamine or cysteine, although the effect is not as great. However, if you use very low dose levels you get potentiation.

CHAMBERLAIN: I think you also saw such things as shock when you used excessive doses of porphyrin alone.

VERMUND: That is true. I remember particularly one patient, a young girl of about 15 years of age, who received 1,000 mg of hematoporphyrin intravenously daily and then got radiation to a small area in the inguinal region. She had a widespread rhabdomyosarcoma which had metastasized to both lungs. She died suddenly on the fourth or fifth day after having received only about 700 or 800 r to the local area, but having had 1,000 mg of porphyrin daily for four or five days, as I recall. She suddenly developed convulsions, which may have been due to the porphyrin, although we could not prove it. At autopsy there was no evidence of brain metastases. It is true that if you get up to very high dose levels you get toxic effects, and the oxygen consumption of the animals is then markedly cut down by the porphyrin.

BLOEDORN: I have been working with Dr. Figge in the same school. We treated several patients with differnt doses of porphyrin and X-rays. There are only a few cases, about 15 in all. It has been our clinical impression that with squamous cell carcinoma of the oral cavity and oropharynx, all advanced cases, the immediate response has been a bit better when porphyrin was added. This difference was only for the response under treatment. About the longer term results we do not have a clear answer. In the case of a large ulcerated tumor of the skin, metastatic from a breast carcinoma, we treated the upper half without porphyrin, then gave the patient porphyrin and treated the lower half with the same dose-time. All were documented with diagrams and photographs. No differences were detected. One thing I would like to warn you about: my clinical impression is:

1. The skin really picks up a lot of porphyrin increasing the reaction produced by the rays. This fact could represent a limitation to the treatment.

2. In the treatment of carcinoma of the bladder the mucosa of the bladder could pick up as much porphyrin as the tumor itself. There is no doubt in my mind that in two cases which we treated with porphyrin and X-rays a much earlier reaction developed than in cases without the

drug, obliging us to stop the treatment due to cystitis.

3. In cases where the small intestine is in the area irradiated, it also reacts earlier and stronger than without porphyrin. The mucosa of the small intestine probably picks up large amounts of the drug also.

CHAMBERLAIN: I might say that some patients have been treated at New York University and at Bellevue Center, too, and they have done some labeling of the copper hematoporphyrin with Cu^{64}. They said that they found some localization in a couple of lung tumors that they treated, but I don't know of any other evidence in human patients.

DICKSON: We are particularly interested in the localization in brain.

CHAMBERLAIN: What did you get?

DICKSON: At the moment we are trying to label the porphyrin with iodine, and this apparently is a tremendous problem because adding the iodine somehow alters the substance so that it does not become concentrated in the tumor any longer. The last I heard was that they thought they had solved some of the problems of synthesis of the labeled material.

VERMUND: I wonder if I may make just one brief comment about the side effect from the injection of porphyrin. These patients become sensitive to sunlight, and they may get rather severe reactions if they are exposed to sunlight.

BLOEDORN: We had a patient whom we treated almost a year ago, and she is still sensitive to light.

VERMUND: It is not true with the copper complex porphyrin.

CHAMBERLAIN: I really don't know what else ought to be included in this discussion. Some of the things which might be considered as sensitizers would even include combined therapy with ultrasound. I think we ought to go into things that are more strictly chemotherapeutic agents. I think Dr. Nickson has reviewed this subject.

NICKSON: We have had for several years a rather extensive program of looking at the question of combined chemotherapy as a modifier of radiation response in transplanted tumors. I should like to emphasize initially that the question of experimental design is, I think, of the essence in any work in this area. It is a complex area, and it is very easy to believe that you have a positive result when, in fact, you probably do not.

The animals bearing the tumors must be randomized; the caging must be randomized. The experimental groups should include the absolute controls, the controls with the chemotherapeutic agent alone, with radiation alone, and with combined radiation and chemical agent. It is important to have large numbers of animals, larger than many of the papers have had in the past in the various experimental groups.

The question of choice of endpoint is very difficult. One can take size, one can take rate of growth, one can take permanent disappearance of the tumor, disappearance at a given time, or histologic criteria. There are other endpoints. None are entirely satisfactory. We chose rather arbitrarily disappearance of the tumor at eight weeks, confirmed by histologic examination. This could be criticized as a harsh endpoint. On the other hand, it seems to me that unless you have a reasonably precise criterion of effect you probably are wasting your time.

We used four different tumors, three in mice and one in the rat, and large numbers of animals. The chemical agent was given as a single injection, a few minutes before irradiation was given. The materials used were TEM, 8-azaguanine, A-methopterin, cortisone, N-methylformamide, and azaserine. In no instance did we find any evidence of additive or synergistic effect with the single exception that with TEM there was an additive effect with sarcoma 180 and with the RC mouse mammary carcinoma.

CHAMBERLAIN: Additive but not synergistic?

NICKSON: Additive but not synergistic. For a time the 6 mercaptopurine work looked as if it were showing an additive effect or even synergism. We ran several thousand animals, and we reached the conclusion that it is, in fact, not even an additive effect. In the course of this we ran into the fact that sarcoma 180 of itself presents a broad spectrum of response to the chemical compound, and this was the thing that was leading us down the garden path. This variable was giving us fallacious results which were cleared up only by being very careful about the pedigree of the individual tumor that we were using.

I think it is only fair to say that these results can perhaps be regarded as valid for this time and station only. An infinite variety of injection schedules and radiation schedules could be set up. It was our feeling, both in terms of the amount of work to be done in getting through a preliminary evaluation and in terms of the likelihood of showing something up, that a single injection and a single exposure to ionizing radiation ought to reveal synergism if it existed. The sad conclusion is that in our hands, under the conditions of our experiment nothing has shown up except with the TEM.

KAPLAN: I believe that Dr. Kligerman has some recent data which are interesting.

KLIGERMAN: This project in which I am involved started as a study by Dr. Daniel M. Shapiro, who is a member of the surgical staff at Presbyterian Hospital.

I might say very briefly what Dr. Shapiro's original idea was. Although there are no known qualitative differences between tumor cells and cancer cells, he wanted to try to exploit the known quantitative differences. Furthermore, most of the chemotherapeutic agents employed had been general cytotoxic agents, such as TEM, 6-MP, and 8-azaguanine. Shapiro wanted to explore the quantitative differences that exist in regard to the quantity of certain enzymes in normal cells as compared with tumor cells.

He started out by working with these enzyme systems which utilize vitamin cofactors for which there exist well-known antivitamins. For instance, he worked on the pyridoxine level in various normal tissues as compared with that in tumor 755, a mammary adenocarcinoma of the C57BL strain mouse. Since then many other substances have been investigated, the idea being that the tumor might have a low concentration of enzymes or vitamins as compared with normal tissues. It is true that some normal tissues might also have a low concentration of one or two enzymes, but in the tumor there was an over-all pattern of low concentrations of these various substances. He was able to demonstrate inhibition of tumor growth employing the appropriate antivitamin singly, and could magnify the effect by more than an additive amount by employing them in combination. Furthermore, the dose of each one of the antimetabolites could be reduced below a toxic level when used in combination, without loss of the inhibitory effect.

In teaching medical students how X-ray works, I had been favorably impressed with the neat explanation that it is antienzymatic in its action, at least in part. Whether this is valid in vivo or not, I do not know, but at least it was one of the explanations that I offered. Therefore, when I heard about Dr. Shapiro's work with antienzymatic substances, I thought we could try to combine them with X-ray treatment. At first, we had statistical evidence that we were getting somewhere, but it would only be of interest to a statistician for the first couple of years.

MOSES: When do we get our turn?

KLIGERMAN: I am sorry. Dr. Shapiro was ready to throw out the whole business because he was just looking for the tumor to go away. Using other standards, we did pick up changes but they wouldn't be of interest to this group, especially the clinicians who want to see tumors go away. However, Shapiro began trials with a new substance called 6-amino-nicotinamide. For the first time, using this compound alone, albeit in quantities which caused a considerable loss of weight in the host animal, he was able to get regression of the tumor. But the amount needed for regression was too toxic. Using a dose which was not toxic, and which was only inhibitory to the tumor, we combined it with 8-azaguanine, desoxypyridoxine, and testosterone propionate. We did a series of experiments having saline controls, sacrificed controls (by that I mean animals sacrificed on the day treatment was initiated), a group treated by chemicals alone, a group treated with X-rays alone, and a group treated by a combination of chemicals and X-rays.

We started treatment 15 days after the tumors were implanted, so that we had what we think were very well established tumors. These varied in weight between about 100 and 135 mg. We obtained that figure from the sacrificed controls.

Several things were observed. All of those animals receiving the chemicals alone showed tumor regression initially. Then there was a leveling off and an escape, so that eventually all the animals died with huge tumors 30 to 35 days after the beginning of treatment. As far as the group treated with X-rays alone was concerned, their tumors didn't start to regress as soon as those treated with chemicals alone but the regression was more prolonged;

tumor size again reached a plateau and then increased in this group. Those tumors treated by a combination of chemicals and X-rays started to regress very early and maintained their regression for a long time. We used weight on the day of sacrifice as one standard of the effectiveness of treatment. In one experiment the mean weight of the tumors on the day of sacrifice in those treated by chemicals alone, even though all of them had initially regressed, was 4, 335 mg. Those treated by X-ray alone had a mean weight of 759 mg on the day of sacrifice. In those treated by a combination of chemicals and X-rays the mean weight was 34 mg. There were 19 or 20 animals in each group. The chemicals were given in five injections over a 6-day period, and the X-ray treatment was given on days 1, 4, and 6.

A summary of the first group of five experiments using 8-azaguanine, desoxypyridoxine, testosterone propionate, and 6-amino-nicotinamide shows the percentage of animals in which there was complete regression at the time of sacrifice, 30 to 35 days after treatment was begun. With the combination of X-ray and chemicals 80 percent of the tumors had completely regressed, whereas with X-rays alone only 33 percent regressed.

We were so delighted with these results that we thought we would like to initiate a clinical trial. We began large animal toxicity tests on 6-amino-nicotinamide. Moreover, since 8-azaguanine cannot be given to patients, we substituted 6-mercaptopurine, and tried it in combination with 6-amino-nicotinamide experimentally to see what would happen.

In more recent data,[17] we again gave five injections of the chemical agent and three X-ray treatments on the same time schedule. These tumors on the average were 16 days old at the start of treatment. You can see that all of the saline treated controls died. You can see that tumor growth in the animals treated by chemicals alone was indeed inhibited, but then there was escape and 19 of the 20 animals were dead at the end of the experiment. You can see that treatment with X-rays alone produced regression followed by escape, and that the average tumor weight at the end of the experiment was 360 mg, with 33 percent of the tumors at the period of sacrifice showing complete regression. Finally, combined treatment with the same dose of X-ray and the same dose of chemicals given concurrently showed an earlier and a more prolonged effect. The average final tumor weight was only 48 mg, and in 80 percent of the animals no tumor could be found at the end of the experiment.

VERMUND: I should like to ask you about the weight curve of these animals. Is it possible that this could be a nonspecific effect?

KLIGERMAN: I think I can answer the question directly. I have the figures on the first experiment with the 8-azaguanine. The weight change at the end of the experiment--

VERMUND: I mean the animal weight.

KLIGERMAN: I have it expressed as the percent weight change of host and tumor. Will that be acceptable, because these animals weighed about 22 grams on the average?

VERMUND: The ones that showed good regression of tumor had lost more weight than the others.

KLIGERMAN: The final average tumor weight in the animals treated with chemicals alone was 3 grams. That is a pretty good percentage of a 22-gram animal. Right? The weight of the host and tumor in these animals treated by chemicals alone increased by 16 percent. Three grams is about 16 percent of 22 or 23 grams. The weight gain in the animals in which the tumor was growing actively after treatment by chemicals alone can be accounted for by the tumor growth alone; thus in effect these animals gained no weight.

With respect to those treated by X-ray alone, the average tumor weight in this first experiment was 900 mg and the weight gain was also 16 percent. This means that these hosts did indeed gain some weight. In those treated by X-ray, plus chemicals, the average weight of the tumor was only 46 mg, and the weight gain was 9 percent. Almost all of the weight gain in this group can therefore be accounted for by an increase in body weight. This was observed throughout the series of experiments.

CHAMBERLAIN: No one has mentioned Synkavite, and I don't know whether anybody wants to or not. I don't have any definitive information on it. We are also leaving out combined therapy in human beings, as you said, so we won't get into things like testosterone, or radioactive phosphorus.

I don't know whether we can really summarize this, except that it seems to me that there are some complex and unexplained effects. You still cannot close the door completely on any one of these three concepts, the abscopal, the sensitizing, or the combined chemotherapy, but with spotty exceptions, none of them to my mind look particularly promising from the clinical standpoint. Here again you are dealing with evidence based on the response of a specific animal tumor in a specific situation, which does not make me too awfully enthusiastic, I must say.

KOHN: If you want a remark about Synkavite, at the nonhuman microorganism end of the scale it did nothing.

KAPLAN: Do you want to specify the microorganism?

KOHN: E. coli and the yeast Saccharomyces cerevisiae.[18]

KAPLAN: I think it would be in order to express the caution that the results described will, of course, have to be extended to other tumors. My particular feeling would be that they should be extended to tumors arising in their original host, because one wants to know whether this is a phenomenon pertaining to a particular host-tumor relationship or whether it is something that has more universal significance.

KLIGERMAN: In answer to your objection, which is to me the same as Dr. Chamberlain's, we have looked at it in this fashion: we think the theory involved, the theory of looking for quantitative biochemical differences as a

means of finding the chemical antimetabolites, which can perhaps effectively augment radiotherapy, will hold for any tumor. This particular combination may not be effective against every tumor. Nonetheless, we feel that searching in this way for something that we know exists as a difference between the tumor and the normal cell is a logical approach as compared, let's say, just to screening.

CHAMBERLAIN: I think what you are saying is that it is useful to look for finer degrees of endpoints in radiobiological animal experiments as well as in human subjects.

KLIGERMAN: Yes. We plan very shortly actually to start in on humans. Our toxicity studies are complete now. This particular combination may not work clinically but this does not mean that the method of finding the chemical agents does not hold just as valid for any animal tumor, whether it be spontaneous or not, or for human tumors.

VERMUND: May I ask you how you are going to design your experiments in human beings?

KLIGERMAN: We have just started to talk about them, but there are two different things that one can do. The one that we as clinicians are most interested in is the long-term project of selecting patients, depending upon the number of groups, either by a flip of the coin or by a table of random numbers. This will be done with each type of tumor, and then treatment will be with X-ray alone or X-ray plus chemicals according to the assigned group. Then we will just follow all patients to observe end results. That is one kind of clinical experiment that can be done.

Another experiment which might give a more immediate effect, although I do not like it as much, involves taking a patient with multiple skin tumors and irradiating some nodules with and others without the chemicals being given. I don't like that experiment for obvious reasons.

FRIEDMAN: What are the obvious reasons? It seems to me that is the best experiment.

KLIGERMAN: I don't think so. I started to do that a long time ago with radiation alone, trying to vary dose. I think all of us have had the experience of treating multiple metastases of a tumor, and each metastasis requiring a different dose to cause it to go away. I think it is a poor experiment.

FRIEDMAN: The differences are even greater from patient to patient.

KLIGERMAN: Not if one is strict about the way the patients are randomly assigned to their groups. In our case the assigning is done by the secretary who first sees the patients, which I think is the only way. It takes longer but it is the only right way to do it. By such randomizing the differences between patients are cancelled out.

KAPLAN: You may find that your experimental design will have changed by the time we finish this discussion.

This may be a heroic effort, but we thought it would be worthwhile at least to consider the problems and the possibilities of evaluating the relative effectiveness of supervoltage therapy as compared, of course, with conventional therapy. It seems best to discuss first of all some of the physical characteristics of supervoltage radiation and some of the equipment that is available.

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IV. POSSIBILITIES OF A NATIONAL EXPERIMENT TO EVALUATE SUPERVOLTAGE THERAPY

1. Physical Advantages of Supervoltage Radiation, and Brief Analysis of Various Types of Equipment Available

Harold E. Johns

In the last few years many new types of equipment have become available and I thought I would summarize some of these very briefly.

Starting at the top of the energy scale are the synchrotron, the betatron and the linear accelerator which may operate in the range, say, from 10 to 100 Mev. In the next energy range we have a number of possible machines, the linear accelerator, the Van de Graaff generator, the resonant transformer and Co^{60} working at a long focal skin distance. At the risk of starting an argument, which no one will settle here, I would say that all of these machines are essentially equivalent from the point of view of radiation, and we might call them 3 Mev machines.

The next range of energy I will designate as Co^{60}, Ce^{137}, and X-ray machines. I am now thinking of Co^{60} and cesium working at a short focal skin distance, and X-ray machines operating at somewhere from 200 to 400 kev. In many ways these fall into the same classification. Then finally we will have cesium working at a very short focal skin distance of not over 15 cm, probably for head and neck work.

I know that this classification is rather arbitrary, but one must be careful to specify, as well as the energy of the radiation, the distance from the source to the tumor because this makes a big difference in the distribution of the radiation which will be obtained.

I think that one of the aims of the radiotherapist is to put a known amount of radiation into a tumor and to put no radiation anywhere else. There are some radiotherapists who might not agree that this is a desirable aim. However, as a radiophysicist it seems to me logical that we want to put the radiation in the tumor and nowhere else if possible, and we would also like to know how much radiation is put there. High energy machines offer a considerable advantage in achieving this aim for a number of reasons. I have a few slides which summarize these reasons.

If you take the percent depth dose and plot it against depth for a betatron you get a dose which is very small on the surface, rises quickly to a maximum at about 4 cm depth, and then falls. Co^{60} is a similar type of curve except that the maximum is now near the surface and the surface dose is higher. At a depth of 10 cm, which is about half way through the average patient, you get something like 50 percent depth dose. This curve for cobalt would equally well apply to the radiation from a Van de Graaff generator or a resonant transformer, and almost to a 6 Mev accelerator, although there

would be a slight increase in depth dose. Two hundred kv radiation produces its maximum dose on the surface, after which the dose falls continuously as you go below the surface.

This slide shows the initial region where the dose starts at a low value and then rises quickly to a maximum. The high energy machines offer a real advantage from a clinical point of view, but provide certain difficulties also, because the radiotherapist must depend much more on physical dose measurements to decide what dose he should give since he cannot rely on skin effects.

I mentioned a minute ago that the idea is to put the radiation in the tumor and nowhere else. If we have soft radiation (200 kv radiation), because of the inherent scattering process at this energy we get radiation coming out sideways and bulging isodose curves outside the beam here which are the result of scatter from the primary beam.

When you reach cobalt energies there is still a slight effect due to sidewise scatter. However, outside the beam there is not too much radiation.

For the betatron, scattering is almost entirely in the forward direction and there is little scatter laterally. This introduces a problem which is inherent in all very high energy machines. When an electron hits a target and produces X-rays, they tend to come out in the direction the electron had before it hit the target. As a result the beam is collimated in the forward direction and you get a much greater concentration on the axis of the beam than out to the side. Consequently you have to put in filters which will cut out the radiation in the middle and allow the radiation to come through at the sides. So, if you like, at this energy we have too much collimation in the forward direction. As you go up in energy this gets worse and worse, and you have to take a lot of trouble to get a compensating filter at the right place in the beam to neutralize the effect. The higher the energy the worse this problem becomes. I think this makes it difficult to operate up at energies much above 25 Mev.

In my opinion the machines in the 1 to 4 Mev region are all essentially the same. Which one is better is a matter of opinion. Which one is cheapest to run is also a matter of opinion. Perhaps the biggest single advantage of Co^{60}, maybe it is the only advantage, over these other machines is its absolute reliability as far as output is concerned. Physicists have found themselves in a terrible fix as a result of Co^{60}, because if they once measure the yield from a cobalt unit and write it down on a sheet of paper and hand it to the radiotherapist, they have no way of getting away from the figure until the source is replaced. Already this has caused considerable concern to many physicists, and it has enabled radiotherapists to write articles in the scientific journals referring to different sized roentgens in various parts of the world. I refer in particular to an article by Dr. Paterson in the British Journal of Radiology last year.[1]

Physicists will have to clean their house and find out how to measure the exposure dose from one of these machines. It even comes into the financial picture because sometimes sources are transferred from one unit to

another, and the person who buys the unit feels that he should pay only for the amount of radiation that is coming out.

When you buy a resonant transformer you buy the machine, you don't buy the yield from it. This problem would not have been brought into nearly as sharp focus if it had not been for cobalt. When you make a measurement on an X-ray machine you write down on paper what it is, but if you change your mind you can always get out of the difficulty later on by saying that the machine has changed, and no one can argue with you.

One of the advantages of the high energy machine is the fact that for the same tumor dose the integral dose is smaller for the high energy machine provided we are dealing with a tumor near the center of the body. If we plot the ratio of the integral dose to the tumor dose against the thickness of the patient and refer everything to Co^{60}, we find that for 200 kv radiation the integral dose is about twice that of cobalt and for 25 Mev betatron X-rays about 0.8 that of cobalt. The ratio depends upon the area of the field, and increases as the field area is made smaller.[2]

CHAMBERLAIN: Do you get different values if you are talking about multiple portal and rotation systems, or do you think the ratio remains about the same?

JOHNS: The ratio is always the same regardless of the number of portals you use. If you calculate the ratio of integral dose to tumor dose for one field, the same ratio will apply to the next field except for the slight difference in tumor depth, so you don't have to consider all the fields that are applied. You just need to consider a typical field and compare the types of radiation on that basis.

Now we come to the problem of the inhomogeneities. In the future I think we should try to correct our dosimetry to take into account inhomogeneities in the body. There is not much point in dealing with hypothetical depth dose data which apply to unit density material and try to carry out the calculations to greater and greater accuracy on such material when in fact we are dealing with a complicated biological system. If we plot the energy absorbed in ergs per gram per roentgen, we find that for muscle and air it requires about 85 ergs/g and is constant for all energies. At low energies the absorption in bone is some 450 ergs/g and in fat about 45.[3] This is something that you are all familiar with for it is the basis for diagnostic radiology. You get a good picture of the chest because of the preferential absorption of the radiation by the bones.

For energies from 200 kv to 10 Mev all materials, gram for gram, absorb the same amount of energy. Finally, at very high energies above 10 Mev, bone absorbs a little more energy and fat a little less but the differences are not nearly as large as at the low energy end of the spectrum. This discussion applies to energy absorption per gram and not per cubic centimeter. If we discuss absorption per cubic centimeter, bone absorbs more energy than fat at all energies because of the differences in density (1.85 to 0.9 g/cm^3).

BOND: How far beyond bone is it before you approach an essentially homogeneous dose again? In going through the rib cage, say, does it require 10 cm or so beyond the ribs before homogeneity is approached?

JOHNS: Definitely, for low energy X-rays, but not so far for a betatron X-ray beam.

BOND: I am just wondering how much advantage do you have with supervoltage when you irradiate through bone? How much does the bone take out with high, as compared to low energy radiations? I realize that right at the site of the bone this is a large and important factor, but deeper in, beyond the bone, how important is it?

KLIGERMAN: It is important, but it isn't cut down by the amount that a piece of bone would take out if it shielded the entire area.

BOND: I realize that but I wonder how much difference it makes.

KLIGERMAN: Dr. Robbins made some measurements.

ROBBINS: Twenty percent at the outside, I would say, in the skull or the pelvis.

BOND: So what we are dealing with is a 20 percent advantage with the high energy beam.

JOHNS: That is for the skull, but I know that in the esophagus it is more than than even with cobalt. When the Co^{60} beam goes through the lung, you can have 40 percent more radiation being absorbed in the esophagus than if those lungs were not there. It will be even higher for the softer radiation. You get the reverse effect coming in through the sternum. I am sorry that I cannot really answer the question specifically.

KLIGERMAN: Granted the high energy radiation is absorbed to the same extent per gram in soft tissue and in bone, in each unit volume of bone in addition to the osteoblasts there are a lot of grams of mineral bone. What happens there?

JOHNS: When you get inside the bone itself you have to take into account the range of electrons that are set in motion. The range of these electrons depends on the original energy of the photon which set them in motion. Some work has been done on this by Spiers.[4] The ionization right next to the bone may correspond to a medium that is composed completely of bone, and then that ionization drops to the soft tissue value if the cavity is a big cavity. If the cavity is a small one then the ionization stays at the level of the value for bone inside the soft tissue within the bone structure. Then you are in trouble because--well, where is the soft tissue? I know that these trabeculae are a complicated problem, depending on the type of animal with which one is dealing.

KLIGERMAN: Do you have any actual information on the betatron, for instance?

JOHNS: No, except that very energetic electrons are set in motion. I think the ionization is very nearly constant through the bone.

My point is that one of the big values of high energies is that we avoid problems arising from the inhomogeneities. Suppose we have an ion chamber placed behind the patient to measure the exit dose. The reading we get on the chamber depends upon the amount of scattering material traversed by the beam, the distance from the scatterer to the chamber, the thickness of the patient, and the size of the field. The reading of an ion chamber will be a function of a large number of extraneous variables. What we would like to have is an ionization chamber which tells us the effective thickness of the patient. We have done this by building an ionization chamber[5] which is about 4 cm high and about 5 cm in diameter. In front of it is a lead plug with many holes in it, all of which are placed at a slight angle so they look back at the source. Such a chamber with this focussing plug in front of it will allow only primary radiation to come through, and will exclude scattered radiation from the patient. So with this kind of a device you can actually make a measurement and determine the thickness of the patient in terms of unit density material. You can replace him, in other words, by unit density material of that thickness, and then you can calculate what the dose would be if the tumor were at the center of it.

A picture[5] of the response of that ionization chamber as the patient is rotated while being treated for cancer of the lung shows fluctuations are real, reflects the rate at which he was breathing, and the curve repeats itself quite remarkably. You can see that the fraction of the primary beam that is transmitted changes from about 15 percent up to nearly 40 percent as the patient is rotated, and it repeats itself over and over again.

CHAMBERLAIN: Will you go back again over what this collimator does for you?

JOHNS: This collimator effectively removes all the variables but one from your measurement, since it only sees primary radiation. It excludes all scattered radiation. It is like a Bucky grid on a big scale.

CHAMBERLAIN: That is the point. It really is only excluding scatter.

JOHNS: It is excluding scatter. Then by measuring the amount of primary radiation that gets through, it tells you immediately what the effective thickness of the patient is regardless of the field size or any other factors. Do you follow that?

CHAMBERLAIN: But with the transit dose method, using lower voltage, you don't really need this.

JOHNS: I think this would be an advantage in every case. I haven't used it on lower voltage but I am going to do it.

ROBBINS: You remember that we hoped to get a device that would measure only primary radiation, but we were limited by mechanical problems, so

that with the largest fields in the biggest patients the reading was grossly untrue and it was a real limitation.

JOHNS: You may place this chamber at any distance and still measure the effective thickness of the patient.

STONE: When you go to lower voltages, as much as 40 percent of your tissue dose is derived from scattered radiation, so in measuring only the primary radiation, you are losing 40 percent of your effective dose.

JOHNS: I am only using the measurement to tell me the effective thickness of the patient.

The second problem that I feel we should aim for in radiotherapy is accurate localization. One of the problems with these high energy machines is that it is difficult, although it can be done, to get a good diagnostic picture.

The third problem, which is perhaps the most important of all, is that we have to clean up our dosimetry to the place where we don't run into the inconsistencies which I mentioned a moment ago, and I don't know how this is to be done. I think the situation is clearing itself up, but unfortunately not as fast as I would like to see it. A lot of physicists are working on it, but it is not an easy problem.

Many of us have been using betatrons for up to ten years now. For the first eight years a Victoreen in a block of lucite was used, and we assumed that this Victoreen would read correctly. We called the doses roentgens. We knew they were not roentgens, but we thought it would measure correctly. About three years ago the Bureau of Standards found in a comparison with radium that a correction factor larger than 1.0 was necessary to make the device read correctly for Co^{60}. The correction depended on the particular Victoreen used and varied from about 6 percent up to about 15 percent. I think this must mean that many of our RBE studies that were done six, seven, or eight years ago (Dr. Quastler did a great many of them, probably more than anybody else) must be out by some unknown factor, depending upon the Victoreen which was used.

At the last Radiological Society of North America meeting Dr. Warren Sinclair gave a talk on comparisons between betatrons at a number of places in the United States, and the doses expressed in "roentgens" varied by some 20 percent from one to another. This clearly indicates that we must express the dose in a unit which means the same thing to everybody. If Sinclair expressed all the doses in rads they agreed within a few percent, I believe. So it is important that we try to get this tacked down and record doses in a true energy absorption unit.

There are ways of doing this now. They are not easy, but I think we must try for this. I should like to suggest that perhaps some kind of a comparison over the whole country would be worthwhile being sponsored. In Canada, we are definitely planning to do this. Mr. Garrett of the National Research Council is going to visit each center with one particular measuring

device and get every hospital in Canada using Co^{60} on exactly the same dose. I hope that they will all agree when he gets through but I am afraid they won't. I think this is one of the things that Co^{60} has forced upon us.

The fourth point which I should like to make is that we must try to confine the beam as much as possible to the region we want, and this brings in the problem of penumbra. A great deal has been said about penumbra. Penumbra arises from the fact that the source is usually not a point, and when you have a diaphragm some distance from the skin you have a region in which the dose grades off. A number of papers[6] have been written on this problem. The penumbra problem can be exceedingly serious in a cobalt unit. It can even be a problem in one of these X-ray machines. Dr. Wootton, the physicist at the Tumor Institute of the Swedish Hospital, Seattle, has just published an article showing that a Van de Graaff generator with an improperly designed collimator produces isodose distributions which are very poor compared to what they might have been if the diaphragm had been designed properly.[7] This is something to which the manufacturers have not paid a great deal of attention, and the radiotherapists have not improved matters because some have argued that the penumbra is an advantage. How they can argue this way I don't know but some have done so.

How do we get around the difficulty? The only way you can get around the difficulty of a penumbra is either to get a point source, which cannot be done even with an X-ray machine, or place the diaphragm near the skin. As soon as you try to put the diaphragm down near the skin, it becomes large and bulky unless you are careful about its design. With such a diaphragm system you may be in trouble when you start to rotate the patient or to rotate the machine around the patient. One way to get around this difficulty is to make the distance from the source to the patient larger. As soon as you do this and then try to rotate you get a monstrous device, which may be so large that you will require a stepladder to see what you are doing when you set up treatment fields.

With these ideas in mind we are building a unit in Toronto. It is somewhat similar to the other rotation units in that it has a counterweight and radiation shield. If you are going to try to solve the problem of a small collimator on one of these machines, the design of the machine must start from the collimator and the patient and work from there. Many manufacturers build a machine and then when they get it finished they tack on some kind of a device to control the size of the beam whereas it should be exactly the other way around. We must start with the patient and make the other parameters of the design satisfactory from the patient's point of view. We are mounting a diagnostic X-ray tube with a small focal spot in the head and completely enclosing it with lead. We can take pictures then to see if we are lined up. If we are lined up the machine is turned on with the source coming across in front of the X-ray tube. In the counterweight we have a remarkable opportunity to put in one of these transmission chambers--honeycomb chambers--connected to the control panel and an integrating dosimeter, so that in each case we can measure the dose under the actual circumstances in which the patient is being treated. In order to cut down the penumbra size we must have the source at a reasonable distance from the skin. I still feel that 80 cm

to the patient's skin is a good treatment distance. If you get it less than that you are losing one of the advantages of the high energy machine. The distance for rotation is about 96 cm.

Fortunately this is going into a new building and the floor is not complete. When the machine rotates around, the head goes 12 inches below the floor. While you are setting up the machine this hole in the floor is covered by a trap door which is only 39 inches below the axis of rotation. So we have a device for accurate localization, a device for accurate determination of dose with inhomogeneities present, and a distance that is large enough to give a small penumbra and a good depth dose for fixed field therapy. By the way, the distance from the axis to the source is not so important in rotation therapy.

The last problem is the design of the collimator. In a photograph[6] of the unit in Saskatoon the outer dimension of the collimator is 8 by 8 inches across and it opens up to a 20 cm by 20 cm field. When the diaphragm is shut down, collimation is provided by a whole series of bars. When you cut down to a 20 by 6 field, the device will go from a 20 cm by 20 cm to a 4 cm by 4 cm field and its outer dimension will be only 8 inches.

I think that this design, which is on a number of commercial machines now, can be improved on. I think it is possible to build a collimator which has very small external dimensions. When it is closed down to the smallest field it looks like that. (Slide) Now if somehow or other we can make this side here just open out in a fan, this side here open out in a similar fan, and this one and this one, and have them connected at the four corners, then as you go to a bigger field the protection out sideways will be less and that is really what you want. You want the best protection for the smallest field because you want a sharp beam. The outer dimensions of this type of collimator alter with field size. In the collimator we are building the outer dimension changes from 10 cm by 10 cm to 26 cm by 26 cm as the field increases from 4 x 4 to 20 x 20. Collimators of this kind are being developed for both our cesium and cobalt units at the Ontario Cancer Institute in Toronto.

KAPLAN: All of us who are radiotherapists are interested in this problem of evaluation of supervoltage therapy quite obviously for its own intrinsic interest. It is worth pointing out that certain granting agencies that have made such machines available to a number of centers are also interested in this question from the standpoint of finding out whether they have made wise investments or not; also, more urgently from the standpoint of determining their future policy. If they have given such devices to some institutions and there is real merit in them, there might then be validity in their giving similar devices to many other institutions. On the other hand, if it cannot be proved that these gadgets have really resulted in a greater number of cured cancer patients at the end of five or ten years, it becomes harder for some of the granting agencies to justify such expenditures. I don't think we are here primarily for that purpose, but it is nonetheless true that part of the interest of one of the sponsoring agencies relates to just this kind of policy question. Whether or not we are going to be able to come up with any answers at this stage is problematical.

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2. Possibilities and Difficulties of Controlled Clinical Evaluation of Tumor Response to Conventional Versus Supervoltage Radiation

Simeon T. Cantril, Gilbert H. Fletcher
Robert S. Stone, Milton Friedman, and Henry Quastler

CANTRIL: I think Dr. Johns is to be complimented on being one of the designers of therapy equipment who puts the patient first and then starts to build the equipment. As he says, so often the equipment is built and then you adapt the patient to it. In the same sense when we think of clinical experiments relative to comparisons of results, using radiations of different energies, the patient comes first. Any experiment which we design must be adapted to the patient rather than the experiment coming first and the patient being adapted to it. Dr. Johns also made a very good point in his remark that in evaluating energy ranges we have to take into consideration other things besides energy. There are such other factors as treatment, distance, etc., which put a machine in one or another category of the instruments that we are talking about. From the standpoint of energy alone, the teleradium apparatus was, of course, supervoltage. The limitations of distance and quantity of radium at the present time do not put the old teleradium apparatus in the category of instruments in which we are interested at the moment.

Supervoltage therapy dates from the early 1930s. I think Dr. Stone can be given credit for having had the first experience with it, followed shortly thereafter by experience at CalTech, and then at three or four other places around the country. So since the early 1930s there have been radiotherapeutic centers which have had at their disposal X-ray apparatus with peak energies ranging from 800 to 1,000 kv. I don't know how many of you have had an opportunity to read Dale Trout's very excellent historical summary which was published in the Texas Cancer Bulletin last year, in which he goes over the development of X-ray apparatus from its start to the advent of what we now call medium voltage irradiation and carries it on further to the engineering of supervoltage equipment. He brings out something which those of us who are somewhat younger may have forgotten; that is, when 200 kv became available certain members of the profession were just as alarmed about its possible hazards as others were excited about its possible benefits.

Probably Dr. Stone is the only one in the audience who grew up in the era when X-ray therapy had its maximum voltage around 140 kv. I had my training in X-ray therapy when manufacturers were trying to reach a peak of 180 kv. The present abundance of Co^{60} units and of X-ray apparatus in the 1 Mev to 2 Mev and higher ranges makes us forget how relatively recent all these things are.

I do not think we need a clinically controlled experiment to demonstrate that the physical advantages that Dr. Johns has shown us relative to skin effect, depth dose effect, bone absorption, and lesser volume dose can be of benefit in the treatment of cancer in selected patients. This does not mean

that conventional radiation still does not have its place in the treatment of a wide variety of forms of cancer, but with the wider availability of high energy irradiation it becomes increasingly clear to me, as my experience with high voltage irradiation now extends some 20 years, that the late results in terms of possible radiation injury, after as long as 10 years, make it very incumbent upon us who have the responsibility for training radiologists to make as certain as we can that their concept of the use of the machines is as well grounded as we know how to make it.

The late complications that one sees ten or more years after treatment of these patients do not vary from the late complications one might see in using conventional therapy except that they may occur in deeper structures and therefore may be all the more hazardous. If one were to establish a biometric experiment to compare the relative value of supervoltage irradiation with the parameters that Dr. Johns has put on them (and certainly one might accept apparatus in the groups that he has mentioned) then one would set out to establish whether any one of the methods of irradiation has advantage in the treatment of selected forms of cancer. You might possibly add to this high energy electron beams, if sufficient of these become available.

The ancillary purposes of such an experiment I think might possibly be more beneficial than the direct purpose of the experiment itself. Since I feel that it is established and will be found by such an experiment that supervoltage has advantages in selected forms of cancer, there are other ancillary purposes which would accrue. First of all, it would stimulate more thoughtful use of this type of equipment. It would stimulate better dosimetry. It would certainly stimulate better follow up of patients, particularly from the standpoint of the observation of late treatment sequelae. All of these things might add to better understanding among radiologists in general, and particularly those using high voltage equipment, as to what they are actually doing.

What would be the factors in such an experiment? First of all comes the patient with his cancer; as I said, the experiment must be adapted to the patient, not the patient to the experiment.

The second factor is the degree of therapeutic judgment and experience.

Third is the factor of equipment and the techniques which one might use with such equipment. One thing which I am sure any such experiment would bring out is that there is more than one way to skin a cat. As we sit here and think about the perfections which might be obtained from fractionation and what is the optimal protraction, I am reminded that when I visit some of my colleagues, I see them doing things which sometimes to me are quite foreign. I find that their results are sometimes as good and sometimes better than I achieve, yet they in no way coincide with what others are doing. I think we sometimes lose sight of the fact that no one perfect therapy technique has been achieved.

In the choice of clinical material one proposes to compare patients treated by supervoltage irradiation with those treated by conventional irradiation in the range, I assume, of 200 to 400 kv. I doubt if one could now ask

either the patient or the radiotherapist who has supervoltage equipment at his disposal to treat patients with 250 kv radiation if they have a type of tumor in which supervoltage has potential advantage. That does not mean, however, that there are not large groups of well-documented patients treated by conventional means that are still available to us in the literature and in our own patient material to serve as comparative groups.

The groups of patients chosen clinically should be those in whom supervoltage is presumed to have some advantage. One might suggest carcinoma of the esophagus, the maxillary sinus, the nasopharynx, the base of the tongue, perhaps the more advanced degrees of carcinoma of the cervix, and one might put in also cancers of the tonsils and pharynx.

How can one possibly biometrically analyze what is called radiotherapeutic experience or judgment? I doubt if that can be subjected to biometric analysis, and it is one of the factors in any such experiment that has to be accepted at its face value. But I do not think, with careful selection of documented material from the literature on patients treated with conventional irradiation, that we need to assume that the clinical judgment or the therapeutic experience of those reporters is inferior to those who might be utilizing supervoltage at the present time. In a good many instances it may well be superior.

Would one in such an experiment interject a good many subfactors related to fractionation, protraction, dose, rotation, stationary treatment, etc.? I would think not, in general, but those are details which one must leave to the therapist, and by and large they come under the category of clinical judgment. I might suggest that factors used to combat infection or anemia in certain forms of tumors might in themselves be more important than certain of these other factors which one might employ, such as protraction.

As an example of a class of experiment in radiation therapy, one might briefly cite the League of Nations reporting on the end results of irradiation of cancer of the cervix. This reporting does not concern itself with the details of the treatment of these patients. It evaluates the results of the treatment of one disease by one agent. It has, however, not barred separate reports by the score on various methods of accomplishment in the treatment of cervical cancer. One could imagine the confusion, frustration and stifling that might have occurred in radiation therapy had the original report of the League of Nations set down in black and white how these patients were to be treated. I think the report would have lost its clarity and would probably have been abandoned long ago.

What would be the criteria of the end result in reporting? It would, of course, depend to some extent on the type of clinical material chosen. It would be essential that the diagnosis in all cases be confirmed by microscopic examination of previously untreated patients. All stages of any one disease chosen for treatment would be included in the reporting.

As to whether one judges results by the living patient and, if so, at what time, or whether one judges results by control of the primary tumor alone

since subsequent death may be due to remote metastasis or other factors beyond control of the experiment. I think that could be argued both ways. I personally would rather see any such report based on the living patient and also include any late sequelae, at least to a period of ten years. For supervoltage irradiation it would require a longer time actually to evaluate the possible severe sequelae of treatment. I think that such an effort might stimulate the close follow up of patients so that we would have no so-called indeterminate groups.

These are broad outlines of a possible clinical evaluation, if you will, on a cooperative basis by those interested in supervoltage irradiation.

One might ask who would participate? I think to achieve the greatest good, that everyone should be invited to participate who has the necessary apparatus, because by inviting everyone to participate the educational values of such an experiment are spread wider.

I think perhaps the biometricians would not be entirely satisfied with such a beginning. I think that out of it, however, on a national basis might come a better pooling of information than we have, and by analysis of the factors which would be reported there might come better information on some of these factors which we have found in our discussions are still somewhat nebulous, factors of dose, protraction, etc.

FLETCHER: I cannot but subscribe to what Dr. Cantril has said, and just as an introduction to Mr. Cornfield's design of an experiment, I should like to tell you some of our present tribulations with the one we have attempted. Maybe due to our lack of understanding of biometrics or poor clinical judgment, or a combination of both, we are fizzling into something which can no longer be called a controlled experiment.

Following a meeting initiated by Dr. Stone, in July 1951, to see if it would be worthwhile to have a number of supervoltage units disseminated throughout the country, and if so, what kind, we applied for 22 Mev betatron. Fortunately, it was the original grant request and there are no biometrics in it. So I am safe. I am not going to have to pay the money back to the National Cancer Institute. I don't owe them any biometric experiment.

Anyway we have a Co^{60} unit and a betatron unit. It is obvious that such radiotherapy equipment is too expensive to be widely available. If you are going to go into high energy levels, 22 Mev is so expensive that you have to find out if there is a definite usefulness for it.

Starting from the viewpoint of practical usefulness, if you take 1,000 cases that come to you, and treat them at 22 Mev, eventually you will perhaps learn whether there are any patients whom you would not have treated as well if you didn't have the 22 Mev betatron. Of those 1,000 patients there may be 10 or 15 who have been specifically benefited by this 22 Mev gadget. If you have 100 or 150, that is another story.

Let's see what happens with the first 1,000 patients. We now have about

1, 300 treated with this so-called experimental design. Patients come to you for palliation and they are going to have supervoltage. For what reason? Perhaps because they are relatives of state senators. You tell me that is not really a criterion of usefulness. Yes, it is. It may be a state cancer hospital and the patient may be the wife of a senator or a representative and she has cancer. She will have to be treated with supervoltage. If you have a betatron and she knows that the betatron is a more powerful machine than the Co^{60}, you may have to put her on the betatron if you want to have a reasonable appropriation in the next biennium. You cannot get rid of such cases.

You are going to get another group of patients who may be better treated with supervoltage. For instance, a woman who has recurrence of carcinoma of the cervix. I think you can treat that better with supervoltage than you can with conventional equipment, but those are rare cases. There are not enough of them, and the location of the recurrence within the pelvis is not the same. So, therefore, although there may be decided benefits from the supervoltage therapy, you can never prove it on a statistical basis and there you are. Or there may be no objective criterion truly speaking, as in the whole group of cases of bronchogenic carcinoma. Supervoltage is better because you get less discomfort in achieving a rather vague result. It may be so. If I had a bronchogenic carcinoma and I wanted to be treated, I guess I would have supervoltage, but that is about as far as it goes.

So, therefore, when we get down to it the number of useful cases is small. These are the cases that are suitable for definitive therapy. In definitive therapy you get two types. You get the palliative and curative definitive therapy. Definitive therapy may be for palliation. You have a run of cancer of the esophagus, but suppose you have a very conservative surgeon, a surgeon who will turn over to you all cancers of the esophagus coming to the institution. The best survival you can expect is about five years; therefore you know from the start that you are doing palliation. You may have to give some really radical treatment to achieve that palliation and to see that the patient swallows until he dies. You establish his swallowing and you open the esophagus eventually. This is a great benefit and yet it is essentially palliative therapy.

What kind of criterion are you going to use? Surely not the five-year survival, because no matter what you do 95 percent will be dead at five years. So you have to use something else. You have only a small amount of control of the survival rate because most patients will die from distant metastases which you are not treating. There are occasionally those in whom survival can be prolonged by control of recurrence locally or something of the sort, but by and large the survival of these patients, if you open the lumen, is not determined by what you have done. They really are not suitable tumors for a comparative experiment although they have some value.

You finally get to the curative therapy. In this situation you may not always cure the patient, but there is a reasonable chance of a cure, or there is a possibility that you may considerably extend survival. For instance, in cancer of the cervix one can cure, say, 25 percent of stage III cases.

This is where you really go to work. You use the 22 Mev betatron, or the Co^{60}. You get together with your physicist, if you have one that is interested in it, and you work out two treatment plans from the physical standpoint. That is to say, from the standpoint of volume distribution, there will be three groups of patients. These are, by and large, those who are decidedly better treated with the 22 Mev betatron from a physical standpoint, those for whom the volume distribution is better adapted to the disease with the Co^{60}, and those who from a volume distribution standpoint can have one or the other. That last group, of course, will be of particular interest, because it will be some sort of an RBE experiment.

We want to be practical. We are not trying to design experiments in which money and practicality are of no concern. I mean we are interested in finding out the practical usefulness of the 22 Mev betatron in the field, not in Washington. We want to design the technique and to treat the patients in a way that is practical.

Maybe we are going to treat breast cancer; if so, we are not going to put the patients in a bath of wax to equalize the isodose distribution. That is not our criterion of physical equivalence.

I asked a biometrician from the AEC to come down for a couple of days and help me. He designed this experiment and I still have it in my files. I tried to do something with it. Well, you get the breast here, with its external contours, and a tumor deep inside. It is not easy to shape the field with the Co^{60} beam but it is possible. When you begin to try to shape the field with the 22 Mev betatron, it gets more and more complicated. That is to say, we get away from this particular type of usefulness.

We want to find a place where supervoltage or megavoltage therapy will be better and has some clinical meaning. Before we go to those that are best handled with the betatron let's go to the central group. In the central group we have tumors of the tonsils, the base of the tongue, etc. You can treat these with essentially the same technique on either the betatron or the Co^{60}, using two parallel opposing fields. With the Co^{60} you will get a midline dose, say, of 140. They are always a little more--150. The subcutaneous dose is quite large. With the betatron you get a central dose, let's say, of 170 or 180. You will get a subcutaneous dose of 110 or 120. The end results five or ten years later may be very different. Here you have some subcutaneous fibrosis; here you have a means of avoiding such a late reaction. You see, if we are going to treat a group of oropharynx cancers on the Co^{60} and a similar group of oropharynx cancers on the betatron, we can determine the disappearance of the lesion, we can determine the equivalence of mucositis, and years later see if the group that has done well from the standpoint of tumor may have a greater degree of subcutaneous fibrosis with the Co^{60} than with the betatron. We may find with that endpoint for those who survived after five or ten years that the betatron has been very advantageous. They both might be alive, but some of the Co^{60}-treated cases may have very crippling late changes.

How are you going to randomize? You run into some trouble. These

patients have neck nodes. As soon as you get a neck node which is a little big you have to put wax on top of the skin, and when you begin to put wax on top of the skin you lose the accuracy of your treatment in our experience. All I am talking about is the experience we have had. I am not saying that some people cannot put a patient in a bathtub full of wax and treat him from a distance and cure him. Because we cannot do it does not mean that somebody else cannot do it.

KLIGERMAN: Why not put a plug of lucite in the face of your cone and you will have the same surface buildup while preserving accuracy?

FLETCHER: Than can produce some gaps. You get an irregular surface. There are some that you can get it close enough, but there are some others for whom you just have to make a mold and make a flat surface and treat through it. You lose a lot of accuracy. We had patients in whom the primary was partially missed and not well treated on account of that. So we finally had to give up the randomization. Now we are treating on the betatron only those cases that have no neck nodes, or in whom the neck node is so deep that you do not need to have such problems with the buildup. So we still are treating some oropharynx cases on the betatron but no longer is it any kind of a controlled experiment.

Let's take the cervix. In cancer of the cervix we decided to treat our late cases by using a large dose. We did not feel this should be extended to the early stage II cases in which with our conventional dose levels we are maintaining, for the two stages together, a five-year survival of about 80 percent. We thought that was sufficient not to change the technique radically considering the possible complications that might come later. In the stage III cases, we feel that an increased dose by the whole pelvic technique followed by some modified radium is in order. We want to give in the late stage II cases, 4,000 r to the whole pelvis; in the stage III cases, 6,000 r to the whole pelvis, and perhaps even 7,000 r in those with a frozen pelvis. We thought when we prepared to try this that both the betatron and Co^{60} could give us essentially the same volume distribution, but it was not so. If you use Co^{60} to give a pelvic dose of 4,000 r in four weeks, you will have skin and subcutaneous doses and fat doses and rectus muscle doses of the order of 5,000 r. If you want to go to 6,000 r in the pelvis, these subcutaneous and connective tissue doses will be about 7,000 r. So we said we would try Co^{60} rotation therapy. Well, we did but maybe we did it wrong. We rotated them and we got some very serious complications. We finally switched all of that group to the betatron. Maybe you could rotate and achieve a distribution giving the same dose, but we really had to give that up, and our randomization just fell by the wayside.

Let's take cancer of the urinary bladder. We decided we could do the same experiment since the volume distribution is not too frightfully different between the betatron and Co^{60}. For a long time we sort of randomized. Of course, you have to have patients of moderate thickness. The patient who really was too thick had to go to the betatron anyway. When we had treated 120 cases of cancer of the urinary bladder, we had 100 with a minimum of six-month survival and quite a few living beyond one year. Although it is not

too striking, they seemed to do better on the betatron, to the point that we felt uneasy about treating a urinary bladder cancer on the Co^{60}. If I had such a cancer, I would have wanted the betatron.

FRIEDMAN: With the newer cobalt units you can do better--

FLETCHER: I didn't have one. Everybody is going to have one machine and maybe not another. I was not designing an experiment for something I did not have but for something I had. You can't do abscopal therapy.

Despite being the principal investigator, I don't have enough authority in my department to prevent the bladders going to the betatron, and they are all going to the betatron. We had 342 cervices treated that way as of the first of the year last year. We had quite a few stage II and stage III cases. We will eventually prove or disprove what all this radiation can do in those late stages.

We will have some notion as to the usefulness of supervoltage radiation in the urinary bladder, to add to the data of Dr. Friedman, with the single source technique and we will eventually have a lot of data as to what you can do with the betatron. From the standpoint of practical usefulness we are going to be able to say that out of 1,000 cases, X cases are decidedly better treated with the betatron, Y are treated equivalently, and Z are treated better on the cobalt. We may say what the betatron can do, but we may not say that it is actually essential to do it in such instances. I mean by that the late cervices and the urinary bladders. I will venture to say that using the betatron alone is not sufficient, and I would say that if you are going to have supervoltage you should have one, two, or three gadgets of different energies and the betatron alone is going to give you a lot of trouble. It is only the small and specific groups that you will finally transfer to the betatron. As for the main bulk of your problems you will eventually transfer them to Co^{60} or something in that range.

What I have just told you is a prologue, an introduction to Mr. Cornfield's talk on designing a supervoltage experiment.

KAPLAN: We will go on now and ask Dr. Stone to continue the discussion.

STONE: I was not warned that I was going to talk to you but I think, having been classed as an old man who started with 140 kv, I can talk a little about the past. Actually I entered radiation therapy with 200 kv therapy. I helped to put the first 200 kv machine in Detroit that was used there. My experience before that was very limited. Two hundred kv therapy was greeted much like supervoltage therapy has been recently. The first unit for supervoltage, so-called, or million-volt therapy, was the one at CalTech. Here I should like to bring out one thing, that I think the experience at CalTech was completely wasted on the field of radiology for the reason that they went off on different ideas entirely from any that had been followed before and they didn't find out anything particularly useful. They didn't compare supervoltage with ordinary voltage. They just tried out what they could

do with supervoltage. They did it in ways that were strange to anyone who had done radiation therapy and they neglected all the lessons of the past. So that, although they built up quite a series of cases before they finished, I don't think it proved to be of much value to anybody.

The next machine was the one at Chicago, run by Dr. Schmidt, and he ran it much the same as he did a standard 200 kv machine. The next one to go into effect actually was that at the Swedish Hospital at Seattle. There, I think, they did try to improve on previous therapy techniques. Then we got ours at the University of California Hospital, San Francisco, in 1934.

We tried to set up a controlled experiment and to see if we could tell whether 1 million volts was better than 200 kv. The way to set up a controlled experiment, as we saw it, was to vary only one factor, which was the voltage, and to leave the field size, protraction, fractionation, dosage rate and total dose at the same level as before. We did try it for about a year that way. But, as Dr. Fletcher said, you start to treat these patients and then you think: Why do I treat such a big field with this radiation? I don't need it. So the first thing you know you are cutting down the field size a little. Then you think: Maybe I could give a little more radiation with this energy than I did with the other. So you increase the amount of radiation. Before you are through you have changed so many factors you don't know whether it was the changing of the protraction, the fractionation, the total dose, or the voltage that gave you your results. I hope that we can come up with some solution for this, although I am rather pessimistic.

We tried to compare the first 35 cases of carcinoma of the cervix that we treated on 1,000 kv with the last 35 that we had treated on 200 kv, and the 200 kv turned out to be slightly superior to the 1,000 kv. This was on five-year end results. This brings up the point: What are you going to use for an end result when you are talking about the value of supervoltage therapy? I think when you go to cobalt and certainly when you go to the betatron and synchrotron, you have to go to different field sizes and you have to go to and entirely different scheme of treating, because your physical distribution is different and if the machines are to be of any value to you, you have to take advantage of their physical advantages. Otherwise, you might as well go on with the machine you had before. But the minute you start to change all of these things then what have you to decide on at the end when you are through? It is whether your patients lived longer, and if you have changed fractionation, protraction, and all of the rest of it, you have nothing then as a basis for the reason your patients are living longer.

I personally think that the greatest advantage of any machine, beyond the so-called ortho-voltage range, is that it makes the radiologist pay more attention, and that is a factor you cannot take into account. It seems that when you get a cobalt machine and still more if you get a betatron, you start to study: What is the physical distribution of radiation? What does the radiobiologist have to tell us? What are the biological factors? Before, we just stuck the patients under the machine and treated them. The radiologist with the high energy machine is paying more attention to business, he is watching what he is doing, he is trying to get more results, he gives his patients

more antibiotics, and he does everything else to keep them doing better. Is it the machine or the attention that he is giving them that is bringing the results? I don't know how you will ever figure that out.

My own feeling is that only the matter of skin saving has been an advance between the 200 and the 1,000 kv radiation, and that the skin saving was probably more than half done when you got up to 400 kv, if you put in a lot of filtration and had a really big half-value layer. The rest of the treatment you have to accomplish in some other way. I think that if we vary our protraction and our fractionation as well as energy, we are not going to know what changed the results. We do know that protraction and fractionation have a definite effect on end results.

I was trying to think what would be the result if all the radiologists in the country had been made to study the science of therapeutic radiology and radiobiology and still go on using 250 kv machines. Would there be as many cures of cancer as there are with all the other high voltage machines we have now? I don't think you would be able to detect the difference. I am told that the results in Paris in the 1920s in the treatment of cancer of the cervix were higher than anybody has reached since that time. Yet we have all kinds of machines, cobalt, and radioactive isotopes to play with. Are they all just methods that we are playing with because they are new?

There are certain physical advantages in the megavoltage beams, however, which I don't think have been sufficiently brought out today because they are so obvious. We are currently treating three women with our 70 million-volt machine, in whom I am sure we could not have reached any satisfactory dose at all with orthovoltage X-rays. They are 300 pounds, 5 foot 4 or 5, and have carcinoma of the uterus. We can put as high or higher physical dose in their uteri with the synchrotron as you can with 220 kv in a thin woman. So there is an example of where you can use this machine.

I think, Dr. Fletcher, you should pick all of your big, stout patients and put them on your betatron. I have nothing to suggest that will give you a controlled experiment unless you can figure that you never could have gotten a sufficient dose into them otherwise.

FRIEDMAN: Suppose you had a 100-pound woman, would you be worse off with your synchrotron? You could treat two women at the same time.

STONE: You would have to put some of that horrible wax on.

QUASTLER: You can always direct the beam from the head down.

STONE: You can take a single field and have no effect with this machine for about 3 or 4 cm. The entrance dose is very small but the exit dose is very big.

That is about all I have to say except that some people have asked me, since I feel that the supervoltage machines haven't accomplished very much except to make us pay more attention to our business, why I have procured a

70 Mev machine that goes beyond supervoltage to the megavoltage range. I gather from several people here today that they think even the betatron has gone too far. I think that somebody has to try out these machines because one doesn't necessarily know from theory what will happen in practice. There are other methods of absorption of energy. Although the physicists think they know a lot about how 70 Mev X-rays will be absorbed, I am anxious to try it out and see what happens.

There is the formation of a certain amount of radioactivity. It is very small. The amount of energy that is actually absorbed from any of these beams is terribly small. What you are getting are small doses in small areas. I think maybe something beyond 70 Mev will have to be tried some day. Somebody has to see what will happen, doing it as nearly the same way as they did before, varying as few factors as possible, and watching the results as they come along.

KAPLAN: A lot of people have been itching for a long time to speak. I think that we might throw the session open to others of the clinicians first of all. Who would like to talk?

FRIEDMAN: I think the spectre which has been haunting this meeting, at least for me, is the cancer lesion itself. The variability of the disease itself dominates every investigative technique. I will tell you what I mean by this. A lovely design of an experiment has been described but there may not be enough of each type of lesion in 1,000 cases to get a real comparison. (Referring to Dr. Fletcher's diagram.) Suppose we had 1,000 head and neck cases? That would be considered a marvelous sample. I am preparing for presentation at Quebec at the end of this month a report on 150 cases of head and neck cancer treated with supervoltage rotation irradiation. But I had to subdivide them into 18 different lesions. You cannot compare a carcinoma of the nasopharynx with a carcinoma of the soft palate. You cannot compare a cervical esophagus carcinoma with a carcinoma of the ethmoids. They are different lesions. That makes an average of six cases in each category.

Let's look at cancer of the tongue. From my clinical experience I think there are at least seven different varieties of cancer of the tongue. When I look at my own small group of cancers of the tongue, rarely do I have duplicate lesions that I can compare one with the other.

Even if you had 1,000 head and neck tumors, the unexpected cure of one case would change the technique from a fizzle to a phenomenon.

But what can we do about it? I think there is something that can be done. To begin with, it is a waste of time to include carcinomas of the esophagus. You will achieve as much palliation and a rare cure with 200 kv as you will with supervoltage rotation irradiation. I think we have fairly well demonstrated that already. The same is almost true for carcinoma of the lung.

Let's look at stage I carcinoma of the cervix, as a test object. Radium or radical surgery is the basic treatment modality for such lesions. Supplementary supervoltage irradiation may contribute slightly to the cure rate

among the 20 percent or so of such cases that are extremely radiosensitive. So stage I carcinoma of the cervix is not a suitable type of lesion with which to evaluate supervoltage therapy.

What about cancer of the bladder? General agreement on the classification of this disease will be achieved within the next few years. The radiotherapist must depend on the urologist to help classify each tumor. Since there are at least 20 clinical varieties of bladder cancer, many complicated procedures must be undertaken with each patient: radiographic examinations, cystoscopic examination, removal of a large specimen for histological examination, unified pathological criteria, and finally bimanual examination under spinal anesthesia. All this is extremely difficult to obtain on each patient, but without it the value of any experiment is almost completely lost. After the lesions are classified, many will preferably be treated by surgical methods.

If irradiation is selected, it is probably true that the Walter Reed technique (central intracavitary source) or even some form of interstitial irradiation will arrest many more lesions than external irradiation. It is possible that only advanced cancers are preferably treated with supervoltage irradiation. This means few cures and slight to moderate palliation. In spite of these doleful prospects, bladder cancer is still a comparatively good test object for the proposed experiment.

Let's take cancer of the tongue. It is a difficult lesion to cure with external irradiation. If we should take cancer of the tongue alone and have a committee of experienced radiotherapists classify and describe each category of cancer of the tongue and spread the classification out and say to anyone, "We don't care how you treat it as long as you classify it properly, so that we can compare one group with another," then I am sure there would be some people in this country who would supply us with controls. In other words, they would use the very effective interstitial radium needles and proper block dissection of the neck nodes, and very seriously challenge our ability with supervoltage irradiation to destroy the tumor in the primary site and nodes in an equal proportion of cases. It does not matter how they treat them, if they have them properly classified, we can compare one group with another.

So I make the strong suggestion that we pick lesions like cancer of the bladder, the tongue, and advanced cancer of the cervix, and see what can be accomplished with them. If you classify them properly then it almost does not matter where the lesion comes from or what the modality of treatment is. It is a useful statistical group of lesions that you can undertake to treat.

Cancers of the tonsils and tonsillar pillars are easy to eradicate. They are a little more radiosensitive and less aggressive cancers. Another reason I would suggest study of cancers of the mouth is that you can see and define and measure the entire primary tumor very well, and you can see and measure the neck node distribution, although you may mistake the significance of a lump. It may be inflammatory but we can allow for that.

QUASTLER: I am speaking as an ex-clinician who is not going to do what

I propose you should do. I would do it if I were still practicing because I agree very much with Dr. Stone that better gadgets will not help us nearly as much as additional knowledge about what radiation does. I am quite sure, though, that the biology of a particular cancer is much more important than the biology of a particular therapist in determining the fate of the patient.

The point which is not being brought out quite sufficiently is that high energy machines such as the betatron are precision instruments and, as such, excellent tools to collect knowledge. They could be used to obtain precise answers to questions of optimum tumor doses, dosage rates and fractionation schemes. With machines up to 1 Mev, it is usually not possible to irradiate a region in the depth with a homogeneous dose, at a homogeneous rate. With high energy machines, this can be done--to be sure, at the expense of very considerable labor. From such precisely given treatments, it should be possible to derive relations between response and dose, dose rate and dose fractionation which are much more accurate than those available so far.

Also I should like to put in a word for the controlled penumbra. It seems to me quite realistic to subdivide the potential tumor region into an inner nucleus which will receive a very high dose, and an outer shell which you suspect may contain cancer but which is too large to be treated with a very high dose; this shell you might then treat as a penumbra, to be irradiated with a smaller dose. (Objections from various members.) Well, that is what you are doing anyway with your 200 kv machines. Besides, you might think of the reciprocal vicinity effect as another reason why you might not want to stop your irradiation sharply at the boundary of the tumor region.

NICKSON: The first question I should like to address myself to is the old question of how one does clinical investigations. I should like to tie this in with Dr. Stone's remark which he stated very clearly and I think correctly, and which was supported by Dr. Quastler, that perhaps the 220 or 250 kv unit used with care and thought, and used to its limit, may be a lot better than we really think it is.

On the question of comparing two treatment methods, it seems to me that to do this without prejudice to either class of patients demands that there is no certain knowledge that treatment method A is better, the same, or worse than treatment method B. This is, in fact, what you are trying to find out. Unless you have certain knowledge as to whether they are or are not the same, then I think you not only have the opportunity for a clinical experiment, I would submit that you almost have the obligation to do it, if the question can thus be answered.

Dr. Stone has raised the issue as to whether supervoltage is, in fact, better than 250 kv. He used 250 for this range of 200 to 400 as a shorthand term. I believe that this opinion is probably shared by a great many radiologists. Is this a fair statement, Dr. Stone?

STONE: Yes.

FLETCHER: What is the question?

NICKSON: The question is, is supervoltage better, the same, or worse than 200 kv? I think this is the point that I wish to make. You made the implicit assumption that you wish to compare Co^{60} with 22 Mev photons. You made the implicit assumption that both of these are better than 250 kv. I am returning to the more basic question, are these better, the same or worse than 250 kv? I am leaving aside your opinion and my opinion for the moment.

FLETCHER: May I say one word because there may be some misunderstanding as to this design? All the cases that are in that category are cases in which we do not get satisfactory results with the conventional method. That eliminates the oral cavity cancers and it eliminates all the early stage I cervic cancers.

NICKSON; Thank you, Dr. Fletcher, for developing the next phase of my talk. The issue then resolves itself into that class of material in which, for whatever reason, we are not satisfied with what we can do with conventional equipment. It seems to me then that it is not impossible to take the sort of material of which Dr. Fletcher has given some examples and to view it in the light of broad professional uncertainties as to whether supervoltage has any genuine residual benefits for the patient, leaving aside again for the moment the question as to why these benefits occur, to set up a randomized experiment to compare 250 kv used with all the skill and care that the group can give, as compared with supervoltage, also used with all the skill and care that the group can give.

It seems to me that even though these machines have been used for some 20-odd years, there remains a question for which we do not have the numbers as yet. I should like to put forward this line of reasoning for discussion and I should like specifically to ask the biometricians what they think of it. They may answer when their time comes.

KAPLAN: Along the same line, I should like to ask perhaps a naive question. Obviously, the moral burden that is borne by the therapist in submitting cases to an experiment of this kind is a serious one. It would seem that any case that has a reasonably good chance for cure by the radiotherapist, or in which the therapist has a personal conviction that it has a somewhat better chance, or at least the same chance with lesser morbidity with supervoltage, is a type of case in which it is very difficult to justify the type of experiment that we are talking about.

That leads me to wonder whether the kind of case that you have all implicitly discarded is not precisely the kind of case that may be used to conduct this type of experiment using a different endpoint. Why not think for the moment at least about the following type of controlled experiment? Let us compare 200 kv against supervoltage for cancer of the lung, or cancer of the esophagus, or some such type of malignancy in which we know that death is almost entirely due to remote metastasis, where we would use as a gauge the response of the primary lesion, and where we have reason to believe that the type of treatment selected will not injure the patient's chances. It may or may not help him. It will not significantly affect survival in all likelihood. Here we are dealing with a variety of cancer that is so hopeless from the

standpoint of cure that perhaps it frees us from some of the moral problems that we run into in the other type of case.

Obviously, this poses its own special kinds of difficulties. One would have to get autopsies on these cases. One would have to do rather elaborate sectioning, etc. But at least I would like to toss this into the discussion.

FRIEDMAN: As far as I am concerned the cancer of the esophagus problem has been settled. The first 40 cases I treated with supervoltage rotation have died within less than two years, which is poorer than several reported groups of cases with 200 kv. With supervoltage therapy you get a higher percentage of early responses, early opening up of the lumen of the esophagus, but the patients still die so rapidly that you have no information of consequence.

KAPLAN: I am afraid you missed my point. We are not trying to prove the survival value of supervoltage in treatment of cancer of the esophagus at all. We are trying to find a test system in which the efficacy of supervoltage versus the efficacy of 200 kv in treating a specified histological variety of cancer in a specific volume can be compared under controlled conditions without regard to the end results as regards survival.

CANTRIL: What is the purpose of the radiotherapy?

KAPLAN: The purpose of the radiotherapy in this instance is entirely different from what it is in ordinary situations.

CANTRIL: If you were thinking of cancer of the lung you would have very little opportunity to make any valid comparisons by reason of just the biology of the tumor. I think Dr. Quastler stated the biology of the tumor is far more important than the energy of the radiation you put into it. You would have difficulty in really determining what happened except that the patient died.

KAPLAN: No, I disagree with that. I am not a statistician, but it seems to me that one can define conditions and criteria reasonably well. One can exclude all peripheral tumors, and treat only central tumors of the lung. One can take only epidermoid tumors, etc. If you get enough cases, you can set up two randomized series which ultimately should be comparable. It seems to me that these other arguments are self-defeating and continue to put us on a spot where for sixty years we have not been able to answer very many questions.

CANTRIL: I think you have to choose a form of tumor first of all in which radiation may have some objective effect which can be measured. I don't think lung cancer is suitable from that standpoint.

KAPLAN: What about histological sterilization of the primary tumor?

LAMPE: Where are you going to get that data? We cannot get autopsies on patients that die elsewhere, and we cannot keep them until they die just to

autopsy them and find out. So, although theoretically that is very sound, practically we cannot collect that information. The only thing we can do is work on the percentage survival basis.

FRIEDMAN: Another thing about both the lung and the esophagus, in most cases the pretreatment biopsy specimen is minute. You cannot get a reasonable estimate of the histological type other than calling it an adenocarcinoma or a squamous cell carcinoma. The radiograph does not tell you with reasonable accuracy the size of the lesion, and certainly it does not tell you how many nodes are present. So that you cannot classify the lesion and you are not sure what stage the disease is in. The most important feature in evaluating response to irradiation is knowing the size and nature of the tumor you are irradiating.

NICKSON: I think Dr. Kaplan's principle is one that deserves exploration. I am inclined to agree, however, that the choice of lesion is perhaps not the optimal one, because it is so difficult to evolve criteria other than death which you can really apply to a large number of cases.

I think Dr. Cantril has touched upon this. The question of endpoint is a very difficult one. It is attractive to think about situations where radiation therapy has a genuine chance of modifying survival of the patient.

I would continue to feel that if we are to act impartially as scientists we cannot allow our opinions to dominate our action in settings where it is possible to collect relatively valid quantitative data, comparing two classes of treatments. If we make assumptions and act solely on them without checking them from time to time, we are throwing away opportunities to act impartially as scientists, and to offer our patients the fruits of advances in medical knowledge. You have your opinion about supervoltage. All of us in this room do, but when we examine the basis for them, they are frequently just opinions.

DICKSON: I should like to put just one more iron into the fire. I really have no right to get up here because I am one of the unfortunate people who have no supervoltage available at the present time. Secondly, because what I am going to say, as a Britisher, is a suggestion to my American colleagues that here is an opportunity where something might be accomplished. Speaking as a member of the minority, I should like to put in a word for the minority of Dr. Fletcher's list, the groups designated "no objective criteria." I particularly draw attention to the soft tissue sarcoma. It would seem to me that this is an area in which we might accomplish something. This is a comparatively unusual sort of tumor. There is some evidence that radiation therapy will benefit at least a proportion of the cases. There is no set measure of agreement as to the ideal method of therapy. Some people advise preoperative radiation; some, postoperative irradiation. Surely, if one pooled the resources of a large number of hospitals, one might accomplish something in this way. We do not know--at least I do not know--that supervoltage has any particular benefit over conventional radiation in soft tissue sarcoma. We have an idea as to what conventional radiation can do, but I don't think we have enough of an idea really to publish anything. No one has enough experience to tell. So I would just add that this is another possible objective for study.

FLETCHER: I may be talking out of order, but Dr. Cantril has asked a very pertinent question. What, after all, is radiotherapy? It cannot be just a study of some odd way of treating a patient irrespective of the benefit he would derive. You would like to try to conduct some truly biological experiments on human patients. That is what you are talking about. If you are going to do that you must use patients who are hopeless. That will give us an RBE of some sort.

You can use the lung cancers, you can use the esophagus cancers, you might as well put down the soft tissue sarcomas. That is in essence a biological experiment. It is useful and worthwhile. Knowledge is worthwhile per se, and it might be applicable later to something else. You will have to do this on a class of hopeless patients that you cannot cure no matter what you do.

To put in perspective the treatment of cancer, the cancers amenable to radiotherapy are those of the head and neck, the cervix, bladder, breast and the lymphomas. That is about it. We don't use supervoltage for skin cancers.. By and large, I would not regard that as a superior method of treatment. We can very quickly divide these into three groups. You don't cure lymphomas by radiation. You do a lot of good, and you palliate easier in certain areas with supervoltage. If I had a lymphoma, there is no question that I would have have it treated on supervoltage.

In cancer of the breast nobody knows what one is doing with radiation and, therefore, you would never get anything out of such an experiment. I believe there is some place for the use of supervoltage in breast cancer but our surgeon is conservative and unless it is a very good case for radical mastectomy we use preoperative radiation at 250 kv.

Let's take the head and neck and the bladder cancers. The urinary bladder is one place where supervoltage can be used. In the head and neck you get cancers which are very well treated essentially by radium, and the vocal cords by 250 kv.

Then you get the intermediate group where we have failed, and we have to begin here to realize the causes of our failure. Where do we fail? The causes of failure are: (1) lack of control of the primary, (2) the primary may be controlled but the neck nodes are not controlled, and (3) the neck nodes may or may not be controlled, but there is distant metastasis. Also you have deaths from other causes, which is a very significant factor in that group; they are old people. With our betatron we have achieved 80 percent freedom from local disease in advanced oral cavity cancers, yet we have not had appreciably better survival.

When you take the gynecological cancers, you get the same thing. With early stage I cancer of the cervix, early cancer of the vagina, and early carcinoma of the fundus, the results of conventional treatment are excellent. There is no reason to change them. Then, of course, patients come in to you with a frozen pelvis or distant metastases and stage IV cancer of the cervix. There is nothing you can do. You might use them if you want to do some biological experiments. Then you get the stage II and stage III cervical cancers, where there is a chance that there will be an advantage with supervoltage.

Unfortunately, when you begin really to look at where supervoltage may have something to offer in the over-all cure rate and freedom from disease at the primary site, they are not too common. This is the place where some sort of program could be started. One could set up some sort of League of Nations or international classification, where one needs more detailed data in these various categories, and observe end results of careful supervoltage therapy in such cases on a cooperative basis, because none of us get enough of them.

This is not the only problem. There are some fringe benefits. I should like to see an experiment set up just on cancer of the breast by which you are going to combine 250 kv with supervoltage. When you mix a drink, if you want a Martini you know how much gin and how much vermouth to use. You should know how much of the 250 kv X-rays and how much of the Co^{60} will give you optimum freedom from chest wall recurrence, not just whether supervoltage is better than 250 kv.

We should also study cancer of the bladder. It would be nice to have some experiments on the Walter Reed technique versus supervoltage. So here are one, two or three groups where we could start and do something which would be classified along the line of the international cervix data.

KAPLAN: Time is moving along. There are a few more reticent clinicians who haven't said very much. I wonder if I can prod them into opening up a little.

LAMPE: I don't have too much to say because it seems to me that things have been covered pretty well. I would say, however, that I had not joined the bandwagon on supervoltage as being the answer to the practice of radiotherapy, but now when I hear you talking about starting all over again with 250 kv and trying to design more efficient techniques than have been used in the past, to compare them with what we are doing now with supervoltage, I must admit that I feel a sense of reluctance coming on me. I think I know what I can do with the lower voltage, and I am, frankly, not very much interested in wasting a lot of time and energy trying to sharpen some of those techniques in order to compare them with what I am doing with supervoltage. I am at a place now where I am trying to learn what I can do with supervoltage.

So far as I am concerned personally, although our statistician friends probably will not accept it, I hope that in the future, after I learn something about how to handle supervoltage or high energy beams, I may be able to make some estimates about its comparative over-all value compared with what I have been doing in the past with my 200 kv machine.

I do find, as I said, a reluctance to join in any work that means reexploring the 200 kv field. I am sure that I don't treat in that field with the highest efficiency, but on the other hand I have a sense of what I can accomplish, although, of course, in an individual case I may not be able to say. I am very much interested in learning how to handle my high energy beam in the same way that it took me quite a number of years to learn what I have learned, which isn't too much, about how to handle the other. I think it will take me a

decade to learn how to handle this high energy beam, and from then on I will have to sit back and look at my experience historically and draw some inferences that way. I just would not want to participate in an experiment where I have to start treating once again with 200 and 250 kv, although in the same breath I will say that there are things, just as Dr. Fletcher said, which I will continue treating with 200 kv. I won't even bother trying them with cobalt because with cobalt I am interested in the problem entities. When you talk about early vocal cord lesions, so far as I am concerned it is absolutely impossible to get any better results than you can get with careful 200 kv treatment. So I would keep on doing certain things with 200 kv, but I would not want to start reexploring that field in relation to other entities to see if perhaps I can improve somewhat on my past experience.

STONE: I should like to make an additional statement here because communication, as is the common talk nowadays, is one of the difficult things to get across. When I was talking about 250 kv as against the other voltage I was talking about one endpoint, and the endpoint was survival for five or ten years or whatever period you select.

I think that what nearly everybody is saying is that they find it so much more convenient to use supervoltage that they would not want to return to using 250 kv in a lot of these cases. I will agree wholeheartedly; in my own department almost everything that can possibly be so treated is treated on the million-volt machine by the staff. They don't do much with the 250 kv machine except flood over to it when we have the million-volt machine running too many hours a day. So there are other things besides survival that enter into the problem of radiation therapy.

I was thinking only of the question of what you are going to do to decide whether one is better than the other from the point of view of survival. I think from the point of view of the clinical practice of radiology there isn't any doubt that when you have to treat a large area or go through bone or do other things of that kind you can do it more conveniently with supervoltage than you can with 250 kv, but that has nothing to do with whether you can sterilize the tumor or not.

ROBBINS: I think the only thing I would add is that perhaps the starting point for this approach ought to be an international classification for all the tumors that we are discussing here that compares in scope with the classification for carcinoma of the cervix we now have. If everybody over the country could talk in the same way about urinary bladder tumors, breast carcinomas, even the lymphomas, as we can now talk about the carcinomas of the cervix, then perhaps we would have the common language that we need before we can begin to ask these questions about what voltages and what techniques are in order.

I would say that if a program is to be instituted, it ought first to be on the basis of an anatomical classification of the extent of disease, and of any other bases of differentiating the biologic behavior of these tumors. For example, in the last month I have had a revelation about lung tumors that was

inconceivable to me a few months ago. This came from Dick Chamberlain's institution. Perhaps, Dick, you might want to tell us about this.

CHAMBERLAIN: You go ahead.

ROBBINS: All right. Everybody has tried in every way he knows to determine the best treatment for, and to predict what is going to happen to patients with lung cancer. Histologic grading has failed to be of definite help; nobody can correlate histology with the results of operation or radiation because the result is a 5 percent 5-year survival across the board anyway and you cannot learn anything from it. We have learned very little about how to treat lung cancer on the basis of the anatomic extent of the disease in terms of lymph node metastasis, which does not seem to correlate very well. What has turned out surprisingly to correlate with survival has been the question of blood vessel invasion as judged by looking at three arbitrary sections through the biopsy material at the time of operation. I think a Masson stain was used in addition to the H and E stain. The question of survival was then examined on the basis of whether there was or there was not blood vessel invasion as so defined.

It seems to me, if I remember correctly, that the prognosis in retrospect could have been made with an 80 percent accuracy on the basis of whether there was or was not blood vessel invasion.

LAMPE: This is on the operative specimen?

ROBBINS: On the operative specimen.

So I want to propose again that the first things we need to learn are things such as blood vessel invasion in lung cancer. Perhaps other such correlations will turn up, but most of all we need to have an anatomic and, second, a biologic, definition of what we are talking about.

STONE: I think it might be well to remind the people here that there is an international commission on tumor grading which has been set up by the International Congress of Radiology, and I think some pathological group cooperates. They are currently trying to draw a classification for other tumors what will be like the League of Nations' work.

CHAMBERLAIN: They have had more success with the larynx than with anything else.

STONE: They are having a terrible time, as you can imagine, with any of the categories.

BLOEDORN: The only thing I want to add is that as far as we can see it is very difficult to design a biologically controlled experiment. I would like to see something practical come out of this meeting, and I would propose one other thing that could be done. That is, that we all treat the patients in a definite period of time, say, three, five, six, or seven weeks, so that our experience would be comparable. If possible with the same tumor dose also for the same period.

KAPLAN: In this instance you are interested in a different problem. Of course, this is not a question of supervoltage as much as simply the degree of fractionation.

BLOEDORN: Using, if possible, the same fractionation, to be able, after several years, to compare the experience of different people using either the same machine or different types of supervoltage.

KAPLAN: Dr. Garcia.

GARCIA: I have had no experience with supervoltage and I could not add anything to what has already been said.

KAPLAN: I suppose this is going to come up later, but I wonder if the notion that was originally put forth by Dr. Cantril that the comparison would have to be made between existing data in the literature on 200 kv versus data now being gathered with supervoltage is entirely unworkable from the statistical standpoint? Conversely, can experiments be so designed now as to include institutions that do not have supervoltage therapy and are forced to use 200 kv, so that they do not have these moral qualms? Could one design an experiment in which institutions that do have supervoltage could cooperate, each trying to use comparable kinds of clinical material, the same classification, and so on, and working up their patients according to the same protocols? Is this type of experiment at all feasible?

CORNFIELD: I was going to make some remarks on it. I have written and torn up a half dozen specific outlines as this discussion went along.

FLETCHER: I think I would like to say one thing before you start. We are talking as though we were the owners of the clinical material, about designing and disposing of heads and necks and what not; but they don't belong to us, they belong to the surgical specialist.

CHAMBERLAIN: The large proportion of head and neck patients we see are referred to us primarily, and we decide whether we want the surgeon to see them.

FLETCHER: If you are going to design an experiment, you have to have the material. It varies considerably from decade to decade and from institution to institution. In some institutions the surgeons would take the cream of the crop, so we are talking about something that we cannot completely arrive at, because we do not have the entire say-so as to this material. In some places there is good collaboration; in others there is little. But we are not the only ones to dispose of material or to design kinds of therapeutic methodology.

KAPLAN: I think the point has been adequately made. Let's let Mr. Cornfield go on.

CORNFIELD: I don't want to disagree with Dr. Fletcher, but statisticians are sometimes practical and my own answer to your question is that I

would welcome, personally, any kind of information that can reasonably be gotten. If there is a reasonable possibility of collecting information on the results of therapy in different institutions under different sets of conditions, this is still information. If it is the best we can get, then let's get it and see what we can make of it. There are any number of medical problems in which controlled experimentation is just out of the question. We try to make progress in those situations despite that fact. But at the same time that we collect this kind of information, or consider an experiment in which one hospital uses one form of therapy and another hospital uses another form of therapy, we must be conscious of the fact that, although this may be the best we can do, there are enough uncontrolled variables so that we don't know what we have when we are through.

I don't see how one can answer this kind of question in the abstract. One has to consider the specific situation. There is a famous example in the field of clinical therapy that perhaps I might as well produce now because if I don't I am sure Dr. Moses will when he has the floor. It was an attempt during the war to evaluate different seasick remedies. There are various ways of setting up such an experiment. You can line up the fellows on a single ship and give alternate subjects remedy A and remedy B. That is a little inconvenient, and it was thought at the time that it was much easier to give one kind of pill to all the men on one ship and another kind of pill to the men on another ship and see what happened. It was thought that this was easier and perhaps good enough, since the ships would be travelling together and be subject to the same weather.

I don't remember all the details of the experiment, but it turned out that the ballast was a very important factor and that all the men on one ship were subject to a good deal less turbulent motion than those on the other. It was, therefore, impossibe to know whether the differences in the incidence of seasickness on the two ships could be attributed to the pills or the ballast.

The possible relevance of this experience to an experiment in which one gives one form of treatment in one hospital and another form of treatment in another hospital is clear. Whether it would make a difference in any particular case cannot be answered in the abstract. The only bit of experience I can bring forth at this moment is a set of data that I analyzed not very long ago in connection with some work on lung cancer in women. The question at issue was the distribution of these cases by histologic types. Each of a number of cooperating institutions supplied data on the proportion of women who had adenocarcinomas and epidermoid carcinomas. There was a significant difference in the proportions from hospital to hospital. I don't know why that was. Maybe there was a selection phenomenon or maybe the pathologists differed in their diagnostic criteria. But if we had made an attempt to make a comparison using one form of treatment in one hospital and another form in another, this would have been a variable we might have controlled. Whether it would have introduced a serious error, I cannot say in the abstract.

I have talked a long time, but I have not really answered your question. Maybe you would like to take a try at it, Dr. Moses.

MOSES: I will say only a word or two, although I have a whole lot to say. As Jerry Cornfield points out, the difficulty with evidence of this kind is that it is difficult to say just what either the presence or the absence of any evidence of a better result means due to the fact that so many variables can enter in which you are unable to randomize out.

If we can turn from seasickness to meteorology for a minute, we are all aware of a good deal of controversy over the making of rain by cloud seeding. However, at the present time I don't think there is any clear evidence as to whether it works or not. I know that I am not alone in this view. I could be wrong. But clear evidence on what can be said about efficacy of rainmaking is essentially lacking after all these years.

One approach has been used which is analogous to the kind of proposal that has been made here. We know what the rainfall has been annually in a lot of communities. Let's just seed some clouds over some of these communities and see if they get a disproportionate rainfall. When you get done you may have tested the statistical significance and everything but it is very hard to know just what is going on.

The really proper approach to it is to devise some process which will decide at random which clouds get seeded and which do not and then to see what you get. When you have obtained a lot of evidence in this way, you will have a conclusion that people will have to believe whether it is positive or negative.

The reason that randomized experiments haven't been done in cloud seeding is interesting. You get cloud seeding experiments going on because farmers need water on their land. Cloud seeders are men who have had a good deal of "clinical" experience, and they can size up a cloud that is a little fatter and lower and darker than the rest and say, "Well, let me seed that one." Now if you have tossed a coin and it has fallen heads, not tails, you might say, "No, don't seed that one." But he says, "Oh, no, we can't withhold treatment!"

The upshot of it is that after three to five years there is almost no clear evidence on whether cloud seeding works or not. You can say that it worked here and somebody else will say it did not work there, and the evidence is not clear enough to admit a firm conclusion.

KAPLAN: I think that what you two have said in a more or less nice way is that this kind of an experiment would hardly be worth doing. Comparing 200 kv in one institution with supervoltage in another institution is almost by its very nature doomed to prove nothing. Is that not what you said?

CORNFIELD: No, that is what you said.

I want to ask just one more question. How many people here would perhaps support such a study (I would rather not call it an experiment) and if it showed that 250 kv is superior would believe it?

KAPLAN: It seems to me that one would not believe it simply because the nature of the experiment does not permit one to draw this kind of conclusion. You would believe it if you could be satisfied that the data were valid, but you could not be satisfied as to this from the very nature of the study and, therefore, you would reject it.

FRIEDMAN: Both Dr. Fletcher and I have treated carcinoma of the larynx with supervoltage and have gone back to 250 kv. That is a somewhat significant conclusion on our part.

FLETCHER: As to the nasopharynx I don't have much feeling one way or the other. If you take a certain selective group, for instance, cancer of the base of the tongue, surely you get much greater systematic control of the primary with supervoltage. I would have to be convinced that you can do it with 250 kv as well.

KAPLAN: That is not what we are talking about. You have treated both groups yourselves. Even though you did not randomize them and take all the other statistical precautions that might be desirable, you yourselves know what was done. But if you had done it with supervoltage and somebody else had done it with 200 kv, would you still believe it? I doubt it.

J. R. ANDREWS: Five years ago, with much the same kind of attitude, I would say, as we see here, a group assembled at Oak Ridge. They dignified themselves with the title: The Teletherapy Evaluation Board, and they set about to study the general usefulness of Co^{60} and other isotopic sources used to radiate patients at a distance. The organization of the group was achieved, and cobalt units were certainly developed. Splendid work had already been done in this direction, of course, by Dr. Fletcher's late associate, Dr. Grimmett.

Case forms were evolved, identifying certain kinds of case material, classifying and distributing it, and perhaps even starting the actual study of patients. What has happened to this project? It was in a sense a design, whether you accept the validity of the design or not. It was a correlated experiment in the sense that it was a joint experiment with the participation, presumably, of many cooperating institutions. What happened to that?

FRIEDMAN: It was not a good design.

SCOTT: What happened to it?

J. R. ANDREWS: It fell by the wayside.

FRIEDMAN: They called for help but it was a bit too late. The dosage that they suggested was too small. They left too great a latitude in techniques. They did not attempt to classify the material that they were going to treat; and, with due respect to our Chairman, they picked as the major lesion for observation carcinoma of the lung, about which they could learn nothing.

SCOTT: You are faced with exactly the same problem, in addition to the general lack of enthusiasm for it.

KAPLAN: It is apparent to me that there is grave doubt as to whether an experiment along the lines of evaluation of supervoltage can be conducted, but I should like Mr. Cornfield to see what he can still do about rescuing the subject. I should like to make one point, however, and that is that no one should draw the inference, which I think would be far too gloomy, that one can't do any other kind of experiment. It may be that the experiment to compare supervoltage with 200 kv is not feasible, and I should like to say, certainly for those of us who are in the Radiation Study Section, that we have no firm convictions on this. In fact, one of the purposes of this meeting was to explore the subject. We are prepared to take a negative answer just as well as a positive answer, but that does not by any means signify that another sound and meaningful experiment cannot be designed which will help us to learn something else about the field of radiotherapy. I would be most unhappy if a negative outcome on this particular question were to cast such a pall of gloom on the whole group that we would lose heart for doing the other jobs which still lie before us.

KLIGERMAN: May I ask one question of the group? How many radiotherapists are convinced that supervoltage is better than 250?

CANTRIL: In what way?

KLIGERMAN: All right, I will accept any area you want. Let's say cancer of the cervix or any major form of cancer. How many of you right now feel that there is a longer survival? I don't mean five years. I will say an increase in the cumulative survival because that is what we are interested in. It is very important.

FLETCHER: Nobody knows yet. It has to be determined.

FRIEDMAN: I know with respect to testes tumors.

KLIGERMAN: Correct me if I am wrong. No radiotherapist here is willing to come forward and say that he knows that the cumulative survival is better with supervoltage. Let's not limit the endpoint to survival at five years, because Dr. Fletcher pointed out a group that had 80 percent control of the primary at one year, but no better survival at five years because of old age and other factors. So let's not limit our material just to five years. Let's take the whole cumulative survival of these patients.

Will any radiotherapist come forward and say that he has evidence or that he is convinced on any basis at all that supervoltage is better in the control of cervical cancer, in any stage, than 250 kv, or radium and 250 kv, or radium alone?

CANTRIL: I think it will be shown, but I don't think there are enough cases as yet.

KLIGERMAN: This is then just an impression. What we are attempting to do here, I think, is to nail it down so that you will convince me, because I may not have enough material to find out for myself, and also to indicate to

all the other hospitals in the country, large and small, that it is worthwhile investing in some form of supervoltage. We don't have the information right now, but you, Dr. Cantril, are willing to tell me that it is better treated on supervoltage.

CANTRIL: You asked me if I have the figures. I don't have the figures yet.

KLIGERMAN: You don't have the figures. So you would call this an opinion?

CANTRIL: Right, this is an opinion.

KLIGERMAN: But you are unwilling to say?

CANTRIL: I am willing to give you an opinion.

KLIGERMAN: What is your opinion?

CANTRIL: My opinion is that for cancer of the cervix in general, taking all cases into consideration, the survival will be better with supervoltage irradiation.

KLIGERMAN: I see. My point is that this is an opinion and perhaps it will be borne out. However, that opinion may not be borne out, in fact, in a way that will satisfy you as to the best method of treating carcinoma of the cervix. So, therefore, since we do not know, and since treating them with supervoltage is an acceptable form of treatment, do you feel that it is acceptable? If not, you should not be using it at all. You have to assume that treating it with supervoltage is acceptable or else you should not be using it.

FRIEDMAN: He answered the question before when he stated that most of his patients are treated on the supervoltage apparatus.

KLIGERMAN: So, therefore, supervoltage is acceptable. Dr. Garcia has had a large experience in the treatment of carcinoma of the cervix with 250 kv and surely he thinks it is acceptable. We have no basis right now for objecting to treating any particular patient by one method or the other, because if we have a basis for an objection we should not be doing it. We can say that using either energy level is acceptable; therefore, I feel that we can enter into an experiment in which, by randomizing the patients, they can be placed on either of the two units in those institutions that have both such types of equipment.

3. Tentative Design of a Cooperative Experiment

Jerome Cornfield

It says here that I am going to give the tentative design of a cooperative experiment, and even before today's discussion I had not really planned to do that, simply because a cooperative experiment requires cooperation not only in its execution but cooperation in its planning. I don't think there is any biometrician alive who can work in a clinical field and give realistic consideration to the problems and the difficulties without help from those really familiar with them. All of the controlled clinical trials that I know of have developed as the result of a cooperative effort on the part of biometricians, clinicians, and others in developing the plans.

Actually I don't feel that I can contribute anything to a discussion of whether a controlled clinical trial to evaluate supervoltage therapy is or is not feasible. A lot of people are arguing that it is not. As far as I know they might very well be right. I simply am in no position to argue with them, and what I thought would perhaps be useful would be to find some people who had at least a tentative feeling that perhaps the possibilities ought to be explored. Here are some of the problems. Let's see if there is any way of licking them. Then conceivably we could emerge with some kind of judgment as to whether this is or is not a feasible undertaking. So I am not going to address myself directly to the question of whether such a trial is feasible.

What I had originally planned to do was simply to say: Here are some of the questions I think we must answer before we can hope to have a trial. But most of these questions have been raised and some of them have been answered in the course of an earlier discussion. So perhaps the most useful thing I can do is to discuss in a little more general fashion the controlled clinical trial, what it is, how it has worked in some fields, what its philosophy is, and then if there is any time we can see to what extent these ideas can be applied to the question of supervoltage radiation.

I think perhaps the first thing it might be worth my saying a little about is the distinction between the kind of study that Dr. Cantril, for example, proposed, and the kind of study that most people have in mind when they talk about a controlled clinical trial. Now I would admit that when I said earlier that I thought the kind of a study proposed by Dr. Cantril would give us some information that we now don't have, I was not entirely enthusiastic about it. If, in such a study we observed a difference between 250 kv and, say, 22 Mev, this difference might be due to the actual effects of the different energies administered, or it might be due to the fact that some institutions that administered 250 kv were getting different kinds of patients from institutions that were administering 22 Mev. Thus, while such results would be interesting, it would be hard to know exactly how to explain them.

I think the best way to illustrate the kind of thing that bothers me is

perhaps to spend a little time on a clinical problem that is now happily settled. This was the possible etiologic agent in the development of retrolental fibroplasia in premature children. I did not come here prepared to discuss this, so that I may not have all the facts ready. But I did look through the literature once and the story goes about like this:

About 15, perhaps 20, years ago suddenly retrolental fibroplasia blossomed out as a clinical entity and lots of premature infants were developing it, and nobody quite knew why. In reading the literature, a purely observational study was made, and I think it was by George Kinsey (in any event, it was a Kinsey but not the Indiana University Kinsey) in which he sent questionnaires out to a number of hospitals that were reporting cases of RLF. It was a set of questions designed to develop some insight into what had changed in their practice.

His article had, as I remember, well over a dozen tables, and for most of the things that he tabulated the effects were completely negative. There had been no change. Three things had changed over the course of this period. One was the use of vitamin drops. One was the use of some form of iron therapy. I cannot make it any more precise. Both of these showed a very close correlation with the development of RLF. The third thing was the development of high oxygen therapy. This was the least closely related of the three. Loads of people read these articles, and reports started to appear in the literature (I think there was a report from Australia) in which some compared hospitals that were using high oxygen therapy with hospitals that were not using high oxygen therapy and they reported a higher incidence of RLF in the former group of hospitals. Then there were reports from people who had discontinued the use of high oxygen therapy on the basis of this observed correlation and reported a drop in the incidence of RLF.

Finally, there were some animal experiments, I think by Patz in Baltimore, in which he actually induced something very much like RLF in experimental animals with high oxygen therapy. In a sense this was all observational. It all pointed toward the fact that high oxygen therapy was the agent, and one would have thought that possibly the matter would have been considered settled at this point. The fact is it was not. Two randomized clinical trials were conducted, in which on a random basis children were assigned either to a high oxygen or a low oxygen therapy regime, and for the first time to everybody's satisfaction it was demonstrated that in fact it was the high oxygen therapy that was causing RLF.

Now this is the kind of situation I have in mind when I say that certainly one can learn things from observations in different hospitals, but if one really wants to be sure that certain things are under control there is no alternative to the randomized clinical trial.

Some remarks that were passed at one of the many meals that I have eaten since I arrived here leads me to say that the well designed clinical trial is perhaps a necessary condition for drawing conclusions, in which you know that certain variables are subject to control, but it is not a sufficient condition. You can have a well designed clinical trial come out with the

wrong answer. So that the controlled clinical trial is a necessary condition perhaps, but certainly not a sufficient one.

I wish I had a much longer list of clinical situations in which the controlled clinical trial had been applied. I didn't really come here with the intention of discussing this, but one of those that occurs to me is the evaluation of streptomycin therapy in tuberculosis. I understand from people who were in on this problem in the days when streptomycin was first introduced that there was a good deal of confusion, uncertainty, and controversial opinion as to whether it was efficacious or not. It was a randomized trial by the British Medical Research Council with several hundred people in the two groups that produced pretty clear evidence of the efficacy of streptomycin. The RLF is another example, and then there are, of course, the recent examples in the evaluation of the efficacy of vaccines--polio vaccine--and the World Health Organization has been studying typhoid vaccine.

What I should like to do now is to mention what I think are some of the leading principles in the controlled therapeutic trial. After having mentioned them and elaborated on them a bit, I might perhaps touch on the possibility of applying these to the question of supervoltage therapy.

The two key words in the idea of the controlled therapeutic trial are first "control" and then "trial." There are a number of things we can specify in advance that we would like to control.

Perhaps the first and most important in the therapy of cancer is the very variable course of the disease. I certainly don't have to tell anybody here how variable a disease cancer is and how difficult it is to predict the outcome in an individual case. I understand that there are well authenticated cases of the complete regression of fairly late stage tumors. How do we control this kind of thing in a national therapeutic trial? The only device that gives assurance of controlling this kind of thing in a well defined sense is the device of randomization. We have skirted around this issue several times and I have no high hopes that I can clarify the situation. A number of people have pointed to the difficulties that arise when one is studying a variable population. But this is exactly the situation, at least so it seems to a biometrician, in which randomization is the appropriate device for controlling the effect of the variability. The reasons for this, I suppose, are essentially the laws of probability. If you flip a coin or draw random numbers or use some such device as that to establish two or more groups of patients, the laws of probability are on your side. These laws tell you that eventually you will have groups of patients that are, in the aggregate, equal no matter how variable the individuals in those groups are. In the aggregate the two groups will tend to be equal, and this is essentially the reason for using randomization in any situation in which it can be applied. I don't think that this is always possible. There are plenty of situations where one cannot, but the principle is that in a situation in which one can, the effect of variables in the population can be ironed out.

A related principle follows almost immediately from the fact that we randomize patients among the treatment groups; namely that we must evaluate

the results statistically. We all tend to form impressions and judgments, but at some point we have the responsibility for looking at our results and really evaluating them on an objective basis and deciding whether any apparent differences could, in fact, have arisen by chance.

There are other kinds of extraneous variables whose effects we try to control in the controlled therapeutic trial. We try to control the effects of supplemental therapy and the institution in which the treatment is performed. We try to control insofar as we can the time at which the treatment is given. If we have a choice we don't compare a treatment given in one institution in one year, with one form of supportive therapy, with another treatment given in a different institution in a different year, with possibly a different form of supportive therapy. If we can help ourselves, we try to eliminate the effects of variables like that.

It may be useful to take just a moment off and indicate what the mechanics are for doing this kind of thing. The way the cooperative controlled therapeutic trial has been worked when there has been a number of institutions involved has been pretty much as follows:

Each institution makes some kind of guess or estimate of the number of patients of the type to be treated that it may expect to see during some given period of time, and it also indicates the variables that it thinks are of some importance to control, the stage of the disease perhaps, the ages of the patients, etc. Then what one does is randomize within those categories. Perhaps a little table will make this clearer. If an institution expects to have 20 patients of whom, say, 10 will be in stage I and the other 10 will be in stage II, then what one does is prepare a set of random numbers, which numbers might appear in this order: 7, 6, 9, 1, 3, etc. The first patient who appears in this group is given the number 7. We might say that all odd numbers get treatment A and all even numbers get treatment B, so that the second patient who appeared with these characteristics would draw number 6 and would get assigned to the other treatment group.

WOOD: Why do you need to randomize if you are going to do it that way? Why don't you take the first patient and put him in one group and the second patient and put him in the next group?

CORNFIELD: That will often be good enough, but if you are going to be careful you might as well be completely careful.

There are some biases that might happen. Let's suppose that the therapist feels that treatment A is, in fact, better. It has not been proven yet, but it is his opinion. He now knows the next patient to come in will get treatment B and the next patient to come in is his patient. He does not want his patient to get treatment B, and so he holds his patient off for a day or two and lets somebody else come in and get the treatment. This may or may not be an important source of error, but if you can alternate your patients you can randomize them just as easily and you might as well.

FRIEDMAN: Can you do that with the limited number of cases you get in

a cancer clinic? Remember that if you are drawing from many different centers you are introducing even more variables. Can't you cut corners and reduce the required number of patients by classifying the lesions into stages and types? Then all you have to know is whether they live or die. It is very simple.

CORNFIELD: Certainly any device for classification that seems to have an effect on the eventual outcome should be used. It was this kind of thing that I had in mind when I suggested this classification by stage. You can substitute any other kind of classification that seems more useful. The thing that isn't controlled unless you randomize is that there are all kinds of possibilities of getting one group of patients who fall within this classification into one treatment group, and another group of patients from the same classification who are nonetheless quite different and who fall into the other treatment group. The only way you can be sure that this is not going to happen is that your classifications are so good that all patients within a classification react the same way, and such a classification scheme has yet to be devised.

There is another kind of control that you find in many therapeutic trials. It is the one that was hinted at just a moment ago. That is the control of the effect of whatever enthusiasm either the therapist or the patient may have for one form of treatment versus another. That is the famous double blind experiment. The notion is really a very simple one. Insofar as the evaluation of the effectiveness of therapy has subjective elements in it, and many forms of evaluation do have subjective elements, then you try to have the person who is making the evaluation not know the form of treatment that the patient received. The evaluation may still be subject to error, but it is not subject to bias.

If the endpoint you use is survival, there is nothing very subjective about survival and this is perhaps not a very important precaution. On the other hand, if the endpoint is whether you can swallow better in cancer of the esophagus, this is a little subjective, and my feeling is that if it is possible to have the evaluation made by someone who does not know the form of therapy it is a good deal safer.

Another key word in this phrase, "controlled clinical trial" is the word "trial." We are getting a trial of a hypothesis that has to be specified with some precision. It is not enough to say that we are comparing treatment A with treatment B. We have to specify the population in which these two treatments are being compared, and we have to specify dosage and a lot of other conditions.

Before the trial is actually undertaken we usually prepare a protocol in which most of the circumstances that will govern the conduct of the trial are written down in advance and agreed to by the responsible investigators.

Now, to what extent can we apply these principles in the field under discussion? I wish I could answer that question. All I can do is touch on some aspects of it that have occurred to me. One question I should like to know the answer to is exactly what we mean by supervoltage and medium voltage

therapy. In a trial, if one were feasible, would one compare 2 Mev with 250 kv? Would it be 2 Mev with 250 to 500 kv? To some extent this decision would have to rest upon the kind of equipment available in the participating institutions. In any event, it is a question that would have to be answered.

A real thorny question, and one that has been touched on by several discussants, is to what extent can we specify the treatment schedule in advance? Let me read to you from one of the cancer chemotherapy protocols, just to give you a rough idea. This is a trial comparing the effectiveness of nitrogen mustard and thio-Tepa in several specific tumors. The appendix specifies:

"Nitrogen mustard is to be given intravenously in a dose of 0.10 mg/kg of body weight daily for an initial course of four days. Thio-Tepa is to be given in a dose of 0.20 mg/kg of body weight daily also over a four-day period by the intravenous route initially. After the original course the patient is to be observed for a two-week period from the time of the last dose. If the white blood count and the platelet count are satisfactory (see below), once-a-week intravenous dosages are given, nitrogen mustard 0.10 mg/kg of body weight and thio-Tepa 0.20 mg/kg of body weight. No drug is to be given if the white blood count is below 3,000 or the platelet count is below 75,000. The dose is to be reduced to one-half of the scheduled dose if the white blood count is 5,000 or below or abnormalities of the bone marrow exist or the platelet count is below 100,000.

Because many of the patients on the study will be outpatients, if it should be necessary to skip a dose by reason of a hematologic depression, an interval of seven days should elapse and the patient should be re-evaluated for the drug at that time. This should apply to inpatients as well in order to insure uniformity of drug administration.

The duration of therapy will be for an arbitrary period of 90 days.

Note: All patients receiving nitrogen mustard will receive 50 mg of chlorpromazine by mouth a few minutes before injection of the nitrogen mustard."

I read this because it seems to me there are two points here. One point is that when you are comparing one treatment schedule with another, it is by no means necessary to keep everything else constant, and clearly in the comparison of medium and supervoltage therapy it makes very little sense to keep everything else constant. If I may become completely theoretical for a moment, it seems to me that in any of these therapeutic trials ideally what we would like to do is not to compare two or more forms of treatment with everything else constant, but we would like to compare the best we can do with one treatment form with the best we can do with another treatment form. This is hard to specify. In some cases it may be completely impossible.

Perhaps if I may take another minute off and refer to a set of experiments that have been conducted by Goldin and his colleagues at the National Cancer Institute just to illustrate this point. Goldin has been conducting a rather systematic study of the therapy of mouse leukemia. He has been concerned

with aminopterin and various other agents. He has not attempted to hold everything constant except the presence or absence, say, of citrovorum factor. Every time he changes his dosage or treatment schedule he finds both the survival time and the toxicity of the administered dose change simultaneously. So the principle he has used is that he will compare different treatment schedules for the same level of toxicity. This is a theoretical concept and it does not have a direct clinical application, but it does at least suggest that to the extent that one has clinical experience, such as the kind that lay behind Goldin's treatment schedule, one attempts to find two dosage schedules which are roughly equivalent in terms of side effects.

To what extent is something like this possible in supervoltage radiation therapy. I don't really know. There are three alternatives that seem like possibilities to me. One alternative (perhaps from the biometric point of view the least satisfactory) is to say that these questions of volume and time distribution and dosage cannot really be specified in advance, and the best we can hope to do is to say that in a trial each therapist will use his best judgment.

This does not seem to me to be a completely satisfactory trial. If this is the best one can do, it would still give some information, but it is necessary to be clear on the kind of information one would get from a trial like this. One could conclude that treatment A is better or no better than treatment B under the conditions of each therapist using his own judgment. It would be hard to specify what this judgment was, and you certainly could not conclude that you would get the same results if different judgments had been used.

The second kind of thing that one can do with respect to dosage in the schedule of treatment is to attempt to build into the trial a study of this question. That is, one could compare treatment A and treatment B, each on several different dosage schedules. I don't regard this as a really practical way of proceeding. The number of cases required to answer even simple questions in clinical therapy is fairly large, and if one tries to vary dosage schedules during the course of a trial one is asking several questions simultaneously, and the number of cases required to answer them goes up very rapidly.

If it is possible it seems to me that the most desirable way to proceed is to pool one's clinical judgment as best one can, to specify in detail as nearly as one can the nature of the treatment schedule and to proceed from that point.

Another question that several people have discussed, and I certainly don't feel competent to answer it, is the nature of the study population. Would one study bronchiogenic carcinoma, as Dr. Kaplan suggested, or would one try to isolate a set of cancers in which one might have some reasonable hope of improving survival by the use of supervoltage radiation? Related to this is the homogeneity of the study population. I suppose that it is possible to subclassify cancer cases in a considerable amount of detail. One can start with the head and neck and have a series of classifications. I don't want to write them all down. One of them would be the larynx. Then one can proceed to subclassify the larynx cases.

If one continues this process and says that one wants a comparison of the finest classification that one can conceive, then one is asking a question that is going to be very difficult to answer. It is going to be difficult to accumulate enough cases in any one of these really fine categories. I think Dr. Friedman was talking about 18 classifications. I will put 1 to 18 down, although I don't know what names correspond to them. At least in some circumstances there are virtues in saying that we will compare treatment A and treatment B separately for each of these 18 classifications, but since we don't have enough observations really to reach any kind of conclusion in any one of the 18, we will look at the differences between A and B, and it may be that the differences are in the same direction for each of the 18 categories, so that there is some virtue in talking about the average difference. So, although we never do make a very definitive statement about the effect of therapy on class 2, one could state that on an average for these 18 classes, there was or there was not improvement.

There has also been some discussion on the measurement of effect. This again is something that I think has been pretty well covered. Certainly the traditional endpoint in evaluation of therapy has been survival time or the proportion surviving beyond a specified period. In a carefully designed trial I think one would almost have to include survival time as an endpoint, and I think there is a great deal to be said for contemplating a really long-term follow-up study in which one evaluated survival time not for five years but for ten years.

Thus, recent work by Sidney Cutler of the National Cancer Institute suggests that for the first five years after treatment the survival outlook for breast cancer was better than the outlook for cervical cancer, but during the subsequent period of time the situation was reversed. So that what you might find out following different forms of cancer for short periods of time might not be the same as you would find out if you followed them for a longer period. This is merely a roundabout way of suggesting that if this is true for different forms of cancer it may be true for different forms of therapy as well, and if you are going to do any follow-up study at all you might as well follow for a considerable period of time. The fact that I have suggested the use of survival time as an endpoint certainly does not preclude the use of any other endpoints that are possible and seem relevant to the question of radiation therapy. Most of the others, of course, tend to have certain subjective elements. One would be inclined, if it were possible, to have this evaluation done by persons who don't know what therapy has been given.

Now I should like to come to my last subject, and the only one I feel any real confidence in talking about. That is how many patients does one have to observe in a clinical trial of this type in order to learn something? Let's suppose that we have solved all the problems that have been raised up to now. We have two treatment groups. We have the percent surviving in treatment group A and the percent surviving in treatment group B, and now we are going to follow one of the first principles laid down. We are going to evaluate this difference statistically. We are going to ask, what is the possibility that the difference in magnitude that we have observed could have arisen by chance? How many cases do we need, speaking roughly, to get us a significant

result? This depends on three things. It depends first of all on how big a difference you are interested in finding. Let's call that Δ. Now if you are interested in detecting quite small effects, of course, you need large numbers of cases. If you are interested only in detecting very large effects, you can get by with very small numbers of cases.

The second thing that this answer depends on is the level of significance at which you are going to test. The .05 level has become conventional. It has become practically illegal to use any level of significance lower than this. This will have an effect on the number of cases you require.

The third thing that will influence the number of cases you require is the chance that you are willing to take of saying that no difference exists when the difference is, in fact, equal to Δ. That is, let's suppose that in fact treatment A will cure 10 more patients than treatment B per 100. At the end of a long trial you observe only a three percent difference, and for the number of cases you studied, this three percent difference may not be significant. If you wish the chance that this will happen to be pretty small, you will need more cases in the study.

Let me give you just a rough indication of the number of cases required. Let's suppose we are interested in a difference of 10 percentage points. We are going to test at the P equals .05 level, and we want the chance of failing to detect a difference of this magnitude to be no bigger than 0.1. Then one would need approximately 440 cases in each of the two treatment groups.

This is a staggeringly large number. What can you do to cut it down? Any science has the inverse square law, and we have an inverse square law in statistics, too. The number of cases that you require, it just turns out, is inversely proportionate to the square of the difference you are interested in detecting. So if you say, "I am not interested in detecting a 10-point difference, but rather in a 20 percent difference," then with everything else the same you will need only one-fourth as many cases (110). If you say, "I am interested in running a trial that will detect a 40 percent difference and never mind anything less than that," then you can get by with 110 divided by four, whatever that is. On the other hand, if you are interested in detecting a 5-point difference you need four times 440 cases, and I won't even put that on the board.

LAMPE: What is the 0.1 again?

CORNFIELD: Let me use another word. Minus 0.1 is what is sometimes called the power. The 0.1 is the probability of failing to get a big enough difference in your sample to say that there is a difference between these two treatments when the true difference is 10 percentage points.

LAMPE: Would you like to reword that?

CORNFIELD: Yes, I will make another try. My hypothesis is that if you could treat a very large number of patients there would actually be, say, 10 percentage points difference between the two forms of treatment. If we now

conduct a small trial, we aren't likely to observe a 10 percentage point difference. We may observe something larger or we may observe something smaller. In fact, we have an even chance of observing something smaller than 10 percentage points. Whatever we observe we are going to test statistically. We may get a difference of 8 percentage points, and on testing that 8 percentage points difference for significance we may say that there is a significant difference. We may on the other hand get only a difference of 2 percentage points and in testing that we may find that this is not a statistically significant difference. We want the chance for that second occurrence to be small, and the chance of that second occurrence is what I have said is 0.1.

JOHNS: Does this mean that there is one chance in ten of this being plus or minus something?

CORNFIELD: There is one chance in ten of my getting a difference that is so small that it will lead me, when I do test of significance, to say there is no significant difference between these two forms of treatment.

WOOD: Isn't that tied up with the P value selected?

CORNFIELD: No, they are different. You see, if there is no difference I don't want to say there is. If these two treatments are the same, I don't want to go around saying that treatment A is better than treatment B. So I introduce this value, P equals .05, to control the chance of my saying there is a difference when there is not.

There is another kind of error I can make. That is by saying there is no difference when in fact there is. Both are errors; they are just different kinds of errors.

KAPLAN: I wonder if this would clarify matters. You referred to this hypothesis that there is really a 10 percent difference. Wouldn't it be clearer if you made the point that if you ran this trial an infinite number of times, it would then turn out by all the laws of statistics working out that you would come out with a mean difference of 10 percent?

CORNFIELD: On an average.

KAPLAN: Yes, and what you are saying is that you want to guard against a specific sample run coming out with such a small difference that you would miss this difference of 10 percent, and you are putting the probability of running this risk at one chance in ten.

CORNFIELD: Yes.

KAPLAN: I don't know if this semantics makes any difference to the group, but statisticians are often hard to understand and I have been exposed to some of them.

CORNFIELD: Dosimetry sounds like that to us.

KOHN: What do you call the second term?

CORNFIELD: What do I call the second type of error?

KOHN: Yes.

CORNFIELD: I apologize for the complexity of what I am going to tell you, but that is called the type 2 error.

KAPLAN: What symbol is associated with the value of 0.1 that you put up?

CORNFIELD: The symbol associated with 0.1 is beta and the symbol associated with .05 is alpha. This is called the type 1 error. That is the error of saying that there is a difference when there isn't, and this beta or type 2 error is the frequency with which you will say there is no difference when in fact there is. In designing a trial you want to control both of them, which is the only reason you take large numbers of cases.

LAMPE: Will you tell me in a very crude way how you go about controlling errors of type B or beta?

CORNFIELD: By taking 440 cases. Let me extend the table by a straightforward calculation. Suppose you want to control the second type of error in such a way that it does not happen more than one time in ten. You are going to do a significance test at the 5 percent level, and you are going to try to detect a difference of 10 percentage points. This requires 440 cases. You say that this is a gamble and one chance in ten is more than one can take. You want to control the type 2 error at the 5 percent level. Then instead of taking 440 cases you will need, it says here, 550 cases. If this is more cases than you are willing to study and you are willing to gamble a little and control this error at the 20 percent level, then it says here you need only 330 cases.

KAPLAN: What kind of inverse square law is that?

CORNFIELD: The inverse square law applies to the difference, not to the absolute values.

Now, of course, the only lesson from this calculation is that if one is interested in detecting small effects one needs large numbers of cases. I don't know whether 10 percentage points is at all realistic. Looking at the results of therapy, it seemed to me that it was not totally unreasonable.

The only lesson here is that for this kind of assurance one institution will not supply enough cases and clearly one needs a cooperative clinical trial.

4. Statistical Discussion

Lincoln E. Moses

I feel like a man who has just come on to recite a poem after a good juggling act. Much of what I intended to say has been said. You may think that some of this is repetitious, and there will be a good reason for it. I was scheduled to talk on high oxygen therapy as a subject for clinical trial, and I think that might be a good place to tie onto.

The basic idea, of course, is that one has a new way of treating a certain class of patients or diseases, that is with high oxygen therapy, and the question that arises is: Is it superior to the alternative treatment? The way to answer this question is to try it and see. That is simple.

But there are a lot of undefined terms. One is what high oxygen therapy is. One is the class of patients or the class of disease where it might be superior. Another is what this control is that it is being compared with. Another is how to try it. Another is how to see it. I guess each of these questions has arisen here in the discussion. Each of them has deep and complicated medical aspects, and each of them I think gets touched by the hands of statisticians at some point.

Let's start with what the class of patients would be. I don't know what class of patients or disease it would make sense to try on high oxygen, but some pointers have been brought up here. First of all, since the theory behind it is that some tumors are refractory to radiation because of anoxia, it would appear that a tumor that is known to be refractory but anoxia has nothing to do with it would not be a sensible candidate. Also from this theory it appears that if the tumor has grown too rapidly for some reason, no matter how much oxygen you give it, this kind of tumor would not appear to be one for which this kind of trial is especially relevant. There may be other aspects. They would have to be thought about and pinned down.

The definition of the treatment is something that Mr. Cornfield talked about. A lot of things would have to be identified as relevant parts of the treatment, such as fractionation, the pressure at which oxygen is to be given (some work has been done at atmospheric pressure with pure oxygen, some has been done at three atmospheres; I guess some could be done in between), the dosage, the anesthesia--all these things are part of the treatment. It would seem that to the extent these could be prescribed and still have the trial be a sensible trial, that is good; to the extent that they cannot be fixed in advance because of things that cannot be anticipated, it would not be good sense to try to fix them. The more that things can be fixed the more precise the conclusions because the more explicitly defined is the treatment about which the conclusions are drawn. But it is not necessary, in order to conduct a clinical trial, to have the treatments spelled out to the last detail.

About the definition of the control, a lot of points of view can be taken. I subscribe wholeheartedly to what was said a few moments ago, that ideally the thing you would like to do would be to compare (with a kind of disease where it is relevant) the best you can do with high oxygen therapy with the best you can do with existing treatments not involving high oxygen therapy. The advantage of using this kind of approach is that presumably you get the most practical kind of answer out of it. The inquiry is addressed to which is more practical to do, one or the other. Thus, if one treatment, as is the case with three atmospheres of pure oxygen, involves anesthesia, I can see no reason at all why anesthesia need also be given with the ordinary therapy, because that is not a part of the best therapy under ordinary conditions.

There are two fundamentally important aspects of statistically sound experiments or clinical trials. Both have appeared in the discussion here, both are relevant, and both should be taken account of. One is the control of relevant but extraneous variables. Randomization is the other. And in a certain measure these two things are alternative to one another.

It has been pointed out that if you try to control too far down, such as an extraordinarily fine classification of disease, eventually you will get into an unworkable condition and you will wait three years to get a case to match up. That is not good. So you cannot undertake, in fact, to control everything. Furthermore, you cannot even in principle undertake to control everything. The reason I say this is that, as many things as you may know that can affect the outcome of the therapy, there are always some things that you don't know. They will be in the papers two years from now or five years from now or ten years from now, and you cannot control them, if you don't know what they are, in the sense of being sure the group is balanced. The only way you can control them is by randomization, which enables you to control them without even knowing what they are, as I think you can see.

Suppose there is some recessive gene which really accounts for how a patient will tend to react to a certain regime if he has a certain tumor. If you actually randomize patients between the two kinds of treatment, then there is a chance, but a calculable chance and a small chance, that you will get a predominance of the recessive gene in one treatment group. As the size of the group increases, this chance gets smaller and smaller. So that randomization enters in as a way of controlling these things. Whether you do or do not know that they exist, it will control them in the long run. So a rough working point of view is to control as many relevant variables as seem crucial and practical and randomize the rest.

A word or two on the mechanics of randomization has already been said. I would like to say a word about one method that can be used. The idea here is that there is much to be said for randomizing at the last minute. Suppose, for example, that when a patient is admitted he is assigned to one treatment. Of course, that is inconceivable. The first thing that has to happen is that a physician examines the patient or, for all I know, many physicians examine him and decide that the patient really can morally, sensibly and medically be treated by either of two treatments. Either one is an admissible treatment for him. There are a lot of patients for whom this is not true. There are

patients who should not get high oxygen therapy because of the condition of their heart, for example. Such patients should not be in the study. It is not meant that they cannot be treated. They cannot be in the study. No one should be in the study who is not eligible for both treatments, because then when you get through you would not have answered the question that you set out to answer.

The question you set out to answer was: You have a patient and you have two ways to treat him. Which one shall you use? One of the things that makes it possible for him to be treated both ways is that he says: Yes, I will go along with it whichever way. In other words, there is a certain amount of volunteering on the part of the patient. A patient should have volunteered to accept either treatment before he enters this study and before one of the two treatments is assigned at random. Some of you may know of the facts that turned up in the polio trials; I don't think it is explained, but it is an undeniable fact that the children whose parents refused to allow them to be used as controls, who refused to allow them to get into the study and said, "No, you cannot use Johnnie as a guinea pig," developed polio less often than the ones whose parents said, "Yes, it is all right with us if Johnnie goes into the study," and Johnnie was given a placebo. There is no reason that one can identify, but it happened. Accordingly you would like to see randomization occur after the volunteering has already been done.

A possible next way of doing this is to take a giant sheaf of envelopes. Finally the time has come. Somebody opens an envelope and in it is a card calling for one treatment or the other. Clearly, there has to be a stack of envelopes for each relevant category. If the stage of the disease is one of the classifications that is considered relevant--

QUASTLER: That is awfully difficult in practice. If you tell a patient beforehand that you might treat him in one of four ways, that you really don't know yet and you will flip a coin, he won't like you.

MOSES: Yes, I agree, this would be fantastically difficult. Maybe Mr. Cornfield can tell us about a practical experience with this scheme. I know it is being tried. My experience does not extend to an actual situation in which it has been used, but it seems to me that just a comment like this would suffice: There is a method involving high oxygen. It involves the risk of convulsions and requires anesthesia. If it is our determination that this is the treatment you should have, will that be agreeable to you? If the lady says "No," then she does not belong in the study because you would have to use her as a control. That is what I mean.

KLIGERMAN: Will you use her as a control?

MOSES: You don't put her in the study because if you put her in the study she would have to be used as a control.

CURTIS: I don't quite understand why this is necessarily true, although I agree it would be desirable if there were an infinite number of patients to draw upon. For example, would it not be possible to decide beforehand that

a person who has a heart condition but otherwise fits well into the oxygen therapy study, could be used as a control. The probability of having the heart condition influence the final outcome would be very low but it would preclude giving the person high pressure oxygen treatment.

MOSES: You will wind up with a lot of heart patients in the control group and intercurrent disease or latent factors associated with heart disease may give spurious results.

CURTIS: You would have to agree beforehand that there are a certain number of situations which have nothing essentially to do with the question involved. For example, a man seven feet tall might be too big to fit in the pressure box but would make an ideal control, but make a rather absurd example.

NICKSON: But a person with heart disease is probably going to die of heart disease before you get to a long survival period, if survival is one of the criteria.

WOOD: How about the second category, people who are just emotionally opposed to having a certain treatment? Is there any a priori reason for not putting them in the control group? I can see that if you had your choice you would not use them, but can you give me a good reason?

MOSES: Yes, I can give you a good reason, but it is a statistical one. It is not medical. There are two reasons. One is just a reason in principle, and it relates to the question one sets out to answer. In the long run a lot of patients are going to come in with this kind of disease. To some of them you could give either treatment. You want to find out, for that optional class of patients, which is the better treatment. If a person identifies himself, "I am not a person you can give oxygen to because I refuse," then he is not a person who can shed light on your problem. He has made a formal objection. The second reason is that you are really not assigning the treatment at random any more. When you really make the assignment at random, you have the laws of probability on your side.

CHAMBERLAIN: I don't know how far you can carry this concept, but there are plenty of clinicians who will tell you that they think the psychosomatic atmosphere has a lot to do with the way the cancer patient responds and with their survival. I mean in this particular instance, the thing that worries me is that the variables you can think of are fine but what about the variables that you cannot think of?

MOSES: That is the reason for randomization. One of the principal reasons for randomization is insurance. If there is a relevant factor that you don't know about because it has not been published yet, you are balancing it with very high probability by the use of your random device.

CHAMBERLAIN: I wonder about your randomization being true randomization as long as we have anything to do with it.

MOSES: It seems to me that is one aspect of it that probably you should

not have anything to do with. Evaluation of the subjective things had better be undertaken by somebody who does not know what the treatment is. It seems to to me that no patient should ever be subjected to randomization except when the physician agrees that whichever way it comes up is all right. "I know which way is better but either way it comes up it will be all right." The randomization should be made by somebody with a table of numbers and the instructions put in a sealed envelope. An approach of this sort won't bias the result. It will slow things up. It may result in your getting findings that you cannot extend terribly well.

For example, just to get something clear that has been talked about, although it seems a little far-fetched: suppose all the physicians decided: "I won't use this on anybody except people 20 to 40. It is all right if you give either treatment to these people." When you get all done you won't have any bias because this is assigned randomly, but on the other hand, your conclusion will apply only to people between 20 and 40.

CHAMBERLAIN: May I ask one more question? We are trying to apply this, I judge, to this possibility of two forms of radiation therapy. What worries me is that it is impossible to do this on a double blind basis because the man who is giving the therapy knows what therapy he is giving. I honestly can't conceive of a proper trial unless you have within the same institution both kinds of therapy and the patients are divided so that the person who is giving one therapy is enthusiastic for the one that he is giving.

MOSES: I go along with at least part of that. You have raised a knotty problem, it appears to me. The first part, that both methods have to be tried in the same hospital, I can only agree with very wholeheartedly. The reason for that is that there are so many factors that are different from one hospital to another: the recruitment of patients, their socio-economic background, the nursing care, the way in which infection is taken care of, the general policy toward transfusion. All kinds of things will be systematically different from one hospital to another. So it would pay to try out the two in the same hospital.

The question of one physician giving one treatment and another physician giving the other, each of whom believing his treatment method will work best, sounds like a choice between evils. Suppose you have one man who is convinced that method A is best and that method B is a lot of nonsense, first of all he probably isn't in the study. He probably said, "I will have nothing to do with it." So there is a fair chance that situation won't arise or at least it won't arise in as acute a form as you put it. There is no question when you get done and you find that method A is better, if you had method B practiced by an indifferent person, it leaves you with the question as to how relevant the conclusion is. One would hope that there would not be participants who had a real commitment to one at the expense of the other of the two methods.

It is something that we should consider. There should be a team in the hospital that deals with these cases. Having two people working on the team tends to break down barriers of this kind. After all, they cannot both be right and I am personally optimistic enough to feel that artificial nonsense tends to

be dissipated by contact. Second, at least the treatment is similar, or very nearly similar, if the same two people do participate in each case.

WOOD: But isn't this valid to the argument that you statisticians have made that you should try to maximize the optimum conditions for both treatment A and B? That is what you have said, and I cannot see how you can have both views. You either have to maximize them or you have to make them the same, but it seemed to me that you have just finished saying that you should make the treatments the same.

KAPLAN: But it seems to me that there is an objection there. To optimize them in the sense that the statisticians were talking about means to optimize the form of treatment as it would be practiced by any good practitioners, whereas the question that Dr. Chamberlain has raised pertains to two specific individuals. The conclusion, therefore, would differ only by the skill and care expended by those two specific individuals. What you really want is a test of validity which could be extrapolated to other places where these two individuals don't exist.

CHAMBERLAIN: May I carry this on to the point of absurdity? I think it has to do with the point. Most of us who practice clinical radiotherapy have gained the impression that the psychosomatic atmosphere that we set up has a lot to do with the way the patient responds, particularly in the serious situations, such as have been described here, and it has to do with the endpoints, too, such as they are, certainly with these palliative endpoints.

NICKSON: We are talking about the curative situation and what you are saying is--

CHAMBERLAIN: Of course, we didn't get very far in trying to specify this, but anyway let me go on with the palliative situation. The degree to which a team transmits its enthusiasm to the patient certainly would have much to do with the patient's willingness. It would have the same sort of influence on the study, I would think. The doctor's enthusiasm or the team's enthusiasm would compare well with the patient's resistance or agreeability to engage in the study.

MOSES: I think that these psychologic factors can have a lot to do with it. But that you should get two different men to do it, the best man in the hospital for method A and the best man for method B, has been supported on the ground that it comes closer to getting the two treatments at their optimum level. It does not violate this notion of comparability, which is why the trial is undertaken. It seems to me that you just can't sacrifice this. There is no point of doing the trial if you are going to get an invalid comparison.

A way of trying to get something like comparability is to use the two people as a unit, and another way of trying to do it is to be blessed with not having the situation arise. This would mean that there would be certain tumors, for example, you would never be trying it on in the sense that there would be certain participants who would not be working on certain tumors, but some place in this plan there would be some radiologists with honest doubt about which of the two methods is better.

CHAMBERLAIN: You are really presupposing a sort of psychoanalysis on this sort of situation, so that you free yourself from emotional bias.

MOSES: No, all I am trying to say is that it seems to me we wish to avoid such an extreme situation as the acute problem you pose of the jolly man whose optimism is so great that it helps to cure the patient with one method and the other whose pessimism is so deep it retards the regression of the tumor of another patient.

NICKSON: As a matter of fact, I think that is also a perfectly good subject for a random experiment all its own: What is the psychic effect of the physician on the end result of cancer therapy?

MOSES: There is a story about a chemotherapeutic endeavor in a clinic. For a long time one of the treatments seemed to be better than its control. Then suddenly it quit being better. Nobody could understand it. They checked back and found that at a certain date they stopped finding it better. "Better" necessarily was based on the subjective reports of the patients. "I feel better." It turned out that the date on which this occurred seemed to be about the same date that they changed receptionists, and the old receptionist had known which patient was on which drug and had given really cheery greetings to the patients on one drug when they came in. "My, you are looking fine"! The first question the doctor would ask was,"How do you feel"? and the patient would say, "Well, I feel fine." This comment was unconsciously associated with the treatment they were on and it certainly illustrates the subjective factor.

CHAMBERLAIN: It isn't silly at all.

MOSES: I know it is not.

KOHN: Won't this error be minimized at least in part by carrying on the trials at a number of different hospitals and then the question of the jolly man cheering as opposed to saddening the patient will tend to average out? We want a randomization of the jollification.

MOSES: I don't know that this particular thing is as important as a lot of other things. You get control when you have both treatments tried in both hospitals.

KAPLAN: You don't know that the jolliness is randomly distributed.

MOSES: I should like to try to point out what I think are four questions which, if solved, would go a long way toward seeing that in principle the question of clinical trials in this area is licked.

One is specification of the class of patients for, in this case, high oxygen therapy, not just which sorts of tumors or which sorts of patients, but what types of criteria you can use on the patient that will eliminate him from the study. What type of tumor is relevant. Criteria for actually saying that this patient is in or this patient is out.

Definition of the treatment as much as there can be agreement, and agreement as to where this is disagreement, on such variables as fractionation, pressure, dose, and things of that kind.

The appraisal of the results. Here I guess I ought to take just a minute for a personal opinion. It seems to me that if one is interested in cure, in the sense of a really increased survival, restriction to hopeless cases is tantamount to wasting time, at least so far as this endpoint is concerned. If the treatment of some tumors gives 40 percent success or 70 percent success, and there is a question as to whether the present method is really as good as the alternative--

STONE: If you took the statistics of the survival of cancer of the lung and and found that they live on the average of six months, then if you took an endpoint of one year, or say nine months, and among those treated the new way you found that a lot more were living at nine months, that would be an endpoint that is perfectly valid and it would indicate that your new treatment was better than the other one. Of course, if no treatment resulted in nine-months survival you would be stuck, but you could pick a closer endpoint than five years if you studied an incurable type of tumor.

MOSES: There are probably some tumors where, if you have a two-year survival figure you have a partial result because there is little difference between the two-year and the five-year survival rate, for example. The point you have raised and the point I just mentioned are both illustrations of the fourth question, which I think is one on which agreement might be sought profitably, and that is fixing the kind of alternatives that the study does intend to undertake.

You will recall that three considerations entered into Mr. Cornfield's basis for giving a necessary sample size. One was the level of significance to be fixed. That is, you wish to do a study where the significance of the test used will be at the 5 percent level, the 1 percent level, or some other level. Second, as an alternative, have a sufficiently large number of cases if you just don't want to miss. This is not a magic number. As opposed to a statistically chosen difference, it is a practically significant alternative or difference. The question that you are really asking is, is there a difference as big as this practically significant one? The third consideration is how nearly sure you wish to be of detecting that practically significant difference, if in fact it does exist. Naturally the sample size will grow if you wish to proceed at a very significant level or if you wish to be fairly sure to detect a practical difference of stated size or if you fix a very small difference as being the practically significant difference you wish to detect. Sometimes the determination of the practically significant difference you really want to detect is very important.

I will give an illustration, but I don't want it to appear to be an implicit opinion on it. Suppose, to use exactly your example, sir, that six months is the average survival in a certain well defined class of lung cancer patients. The question that might be asked here is whether nine months is a practically significant alternative in the sense of its being something that we really wish

to detect. The answer to this may be "Yes"; the answer may be "No."

Another example is that there is a certain tumor, let us say, in a well defined class of patients which yields to conventional methods in 50 percent of the cases. In the case of high oxygen therapy, it takes four hours to give a treatment with the method involving the pressure tank, etc. All right, suppose it were true that the use of that procedure would increase the survival to 60 percent. If this were true, you see, it might or it might not be that high oxygen would become the standard method because of that saving and in spite of its inconvenience. It may be that 55 is a practically significant difference. It may be that with such stringency in the practical application, 70 percent would be the practical goal.

These are not easy questions. They are, however, crucial questions for establishing priorities. If we are going to do a study, which study will we try first? Do we really wish to study this clinical problem for such a small difference if it takes so many observations, etc. ? That is the fourth class of question: (1) The patient and the disease specification; (2) the kinds of treatments, and their specification; (3) the criteria of appraisal, survival or palliation or whatever else is relevant, but the specification of each endpoint and the specification of how that appraisal will be made; and finally (4) the fixing of the objective for the sample size.

CHAMBERLAIN: May I ask another question. Dr. Moses, what sort of horror does this give you? Instead of such an endpoint as this, what if a study were proposed to you in which the endpoint you would have would be whether the patient is turned from a rather uncomfortable to a moderately comfortable patient for six months? You want to go by the percentage of patients who have this kind of subjective response. How would you determine the significance in this kind of indeterminate situation?

MOSES: It does not give me the horrors. It poses a lot of problems. One is how really to tell that one patient, for example, is moderately comfortable and another one is more comfortable than that. I mean it isn't easy. You have to decide first of all if it is something that can be found out. But if you have something that you can actually measure--I don't necessarily mean with 100 percent certainty either, but if you can get it fairly reliably--the lack of reliability will tend to inflate the necessary sample size--if you can determine reasonably reliably the number of people who show improvement in this sense it certainly is feasible to use such an endpoint.

The real problem here is not that it is intrinsically unworkable. It is just that it is difficult to make this kind of measurement. It may require ingenuity and it is conceivable that it might be impossible, but it is also conceivable that it would not be.

QUASTLER: I want to address this question to both statisticians. From all we know about radiotherapy and cancer, the alternatives proposed will result in small differences. A 10 percent difference is what we might optimistically expect from high energy radiation. Doesn't that make one awfully pessimistic about the chances of getting a statistically significant difference at all?

MOSES: That is the type of alternative I really think you might be working toward.

QUASTLER: I am afraid so because neither of the two proposed experimental treatments, the use of oxygen or of high energy, is suspected by anyone of yielding very large improvement.

KLIGERMAN: It is just a matter of taking more cases if you run into the difficulty of a small difference.

QUASTLER: You cannot pick them off a tree.

WOOD: This brings up something that I have often argued against in cellular radiobiology; that is, the use of survival versus the dose-modifying principle. We have talked today about taking risks. There is a reluctance to take risks, to use for our sample population a cancer that would cause, let's say, 90 percent deaths in a year. So few are left at the end of the year that we would not want to study that type of cancer. But take 100 people in your population, at the end of one year there will be only 10 of them alive under one type of treatment. Ninety percent of them will be dead. Now suppose that the alternative treatment, high energy therapy, if you will, is only 10 percent better than the first case. Well, of the 90 who die, 10 percent is 9. Nine more will give you a total survival of 19 instead of 10. So your treatment, if you work with a population consisting mostly of dead individuals, is effectively 50 percent better by one method than the other.

CORNFIELD: The question is what percent die. The fellow who first arrived at the required sample size was talking about a 10 percentage point difference. The answer depends to some extent on what level that 10 percentage points is calculated from. Do I make myself clear? If you want to compare a 30 percent survival with a 40 percent survival, the sample size is not exactly the same as a 90 percent survival compared with an 80 percent survival, but the differences are not large. The important thing is the 10 percentage point difference.

WOOD: But we are looking for ways to assay that 10 percent difference.

CORNFIELD: But you help the assay by saying that it is 10 percentage points difference on a base of 50 percent. This gives the maximum number of cases required to detect a difference of 10 percentage points.

MOSES: What you are trying to say is that it may be only a 10 percent difference but twice as many patients are alive and, therefore, it is important.

CORNFIELD: From the radiation therapy point of view that is right.

STONE: You are wrong in the sense that when we say 10 percent we are thinking that 10 percent will be alive. An improvement of 10 percent on a baseline of 10 percent is 1, not 10.

WOOD: We are thinking only about the relative difference.

JOHNS: When he says 10 percentage points he means 10, but when Dr. Stone says 10 he means 10 percent of 10.

CORNFIELD: We have dosimetry problems in sample size, too. I think that is what it amounts to. The question is, is 10 percentage points the right thing to make the comparison on? Let me extend this 440 table. I said that alpha is equal to .05, beta equal to 0.1, and we had a difference of 10 percentage points. I left one thing out. That was the level at which this 10 percentage point difference occurred. Let me put that in. Actually what I had assumed was a 30 percent survival rate versus a 40 percent survival--no, I am sorry, 35 and 45. That is what gave the sample size of 440. Now I have not gone down to your 90 percent, but I do have 20 percent here in the table. Everything else is the same.

Suppose you want to get a 10 percentage point difference on a percentage of 40, you would need a little more than 440 cases, approximately 500. From a practical point of view a 10 point improvement on 30 is a lot less spectacular than a 10 point improvement on 20.

WOOD: But that is a lot of difference, depending upon your point of view.

KAPLAN: Is this symmetrical around the 50 percent point?

CORNFIELD: It is symmetrical about 50 percent. Suppose I make this 70, 80, and 60. Does that help? The answer is the same whether I am talking about 30 percent or 70 percent, 20 percent or 80 percent. I think all we can really do in answer to this is give a schedule of the number of cases for the alpha and beta and start at this level and then this is as far as our dosimetry goes.

LAMPE: Well, give us the schedule.

KOHN: If you are going, say, from zero to 10 percent alive, that is still going to require roughly the same number of cases in the study.

CORNFIELD: It is going to be a number smaller than 340.

KOHN: A great deal smaller? I think that is the point. Is it going to be a great deal smaller? You work with a disease from which all the patients are dead at the end of a year, and then you try a treatment which at the end of the year gives you a 10 percent survival. That is only an increase of 10 percent the way you are saying it. How many patients would be needed in that kind of a study to establish a statistical significance?

CORNFIELD: Let us compare 80 with 90 percent. Then the number of cases will be in the ratio of 0.8 times 0.2 to 0.9 times 0.1. That is 0.16 to .09. For 90 percent you will need 9/16ths as many cases. If it is zero and 20 percent you can get by with very few. If it is really zero one case is enough to show it, but you don't have zero.

KOHN: I am sure that you can increase the efficiency of your method of assaying by selecting a disease and by working with the disease up until the time that all of the patients are expected to be dead, because then the 10 percent increase in survival, which is only from zero to 10 percent, will require an appreciably smaller number of cases to establish the significance of this difference than if you are working with something like 50 to 60 percent.

CORNFIELD: I understand what you are saying and it is true, but it is not a choice that you really have. You have treatment A and treatment B. Treatment A is going to give a certain percent of surviving patients. We don't know what it is. It may be 30, but you cannot say, "I would rather have it zero."

You can say: instead of studying 10-year survival with breast cancer, where the survival might be 30 percent, I will study 1-year survival with lung cancer and that will give me my zero percent. Then you cannot continue to talk about 10 percentage points improvement in both cases because the 10 percentage point effect may go down to a 2 percentage point effect.

CHAMBERLAIN: I happened to be bemused again by the impact of these high oxygen cases when Dr. Moses was talking about this statistical variation. I wonder if this interpretation is correct: that the reason this makes an impact on us as clinical therapists is because here is a situation, not involving survival but regression of tumors, in which we would expect zero response with any method we have known before and Dr. Nickson reported regression in, was it 7 out of 11?

NICKSON: Something like that.

CHAMBERLAIN: Seven out of 11. In other words, 60-odd percent of these people showed marked regression. Doesn't this fit what you were talking about a few moments ago, that here you are subconsciously applying statistical evidence from the zero point to the 60 percent point and that is why the picture looks impressive?

QUASTLER: I want to tie this in with the statistical problems. The reason why rigorous tests are being used by statisticians is precisely that experience has proven that intuition and general impressions of unorganized experience are highly unreliable. If you doubt it, read the medical books written by the great clinicians 150 years ago. So in order to make progress rapidly, we have to comply with the most rigorous statistical tests.

We have been shown this evening that the material so far collected is not statistically very significant. It does not give us much more than impressions.

It was also shown that in order to attain proper levels of significance, based on differences in percentage survival, we would need an amount of experience which we can hardly hope to accumulate in the foreseeable future.

So, would it not be well to bring the statisticians and the clinicians together and have them search all the possible criteria very carefully for

potential usefulness in an experiment? After all, you can base statistical judgment on more than just survival, and a broadening of our scope seems to be necessary.

KAPLAN: This is a constructive note on which to end a long day in which all of you have been quite active. We will go on with this discussion in the morning, and I intend to bring up the desirability of some actual working conferences of groups interested in pursuing further the question of one or another kind of clinical trial. You might be turning over in your minds, as the result of all you have heard today, your personal feelings as to whether it would be worth further exploration, in which case you will be better prepared to comment when the opportunity comes.

5. Discussion

J. W. J. Carpender

I don't have anything profound to offer. I have been keeping quiet because, as you all know, my experience with supervoltage has been pretty brief compared to a lot of the people here. Like the others, we don't have any conclusive results. We have tried to arrive at certain impressions, and I think the impressions agree with those of the others.

To point out one thing on which there was comment earlier, we were one of the agencies given a large amount of money for special machines. We warned at the very outset that many years would elapse before any definitive answers would be available, and I think our experience has proven that this will be true. After some four years, it still seems to me that it is going to be a rather long time before we are going to get a full evaluation.

I cannot but agree with Dr. Stone in many things he said, and with the other discussants also. Take, for example, carcinoma of the larynx which we are treating on supervoltage. With 250 kv therapy we have an 88 percent 5-year survival if we group the first two stages. We have 100 percent in stage I. We still like supervoltage better, but we are not going to gain any information here at all with regard to survival. I think I am in agreement with Dr. Stone that we are giving the supervoltage patients better general care.

There is one other factor in our use of supervoltage, namely that our ear, nose and throat staff have convinced themselves that it is better. I am not going to go back to 250 kv for this reason, since we are getting cases from the ear, nose and throat staff on the basis of our using supervoltage that we would not otherwise get.

I think it would be nice if all the therapists would give some of their impressions. The best way I can answer for ourselves is to say that in the base of the tongue, the nasopharynx, the bladder, the lung, it is a good deal easier and nicer to treat on the supervoltage, and in the case of the tongue you get in a much better dose.

I am not half as pessimistic about this meeting as the tone yesterday seemed to indicate most of you are. I think that we can have some sort of combined program. Dr. Fletcher pointed out that his experiment had somewhat fizzled. I am sure that ours has too, because we started out in very much the same way, trying to do the same things. I don't think we can do it ourselves. I think we have to get together and have some sort of cooperative study. I think this will get us many of the answers.

I should like to point out that in my opinion there are two approaches that we can take. One is the short term and the other is the long term. I would

use the example that Dr. Kaplan got jumped on for earlier. That is carcinoma of the lung. I think this lends itself very nicely to a short-term program. The statisticians agree that there are many variables that we can neglect if we have to, and in carcinoma of the lung, neglecting a lot of these, I think we can get some short-term answers on a cooperative basis.

The long-term experiment seems to me to be a straight cure rate problem. But I certainly would be in favor of a cooperative program on both of these types of endpoints. In the short-term study with carcinoma of the lung there are a lot of ancillary answers that we can get, but I think survival would be the important thing.

BOND: Would survival be a short-term experiment?

FLETCHER: A very short one for cancer of the lung.

6. General Discussion
(Various Subjects)

KAPLAN: There is one point that I neglected to bring out yesterday; that is, perhaps in some ways the most important argument for trying something like carcinoma of the lung is that any group that is going to undertake an experiment of this sort will really have to learn the ropes.

One might make a pretty good argument for trying a situation like carcinoma of the lung for its educational value. It could be done on a relatively short-term basis so that you learn whatever you are going to learn fairly quickly; second, you have learned in the process the mechanics of handling protocols, and of handling the actual procedures of randomization, and so on, while doing an experiment which may or may not pay off in any significant fashion with regard to survival. You can regard the results of the experiment as perhaps expendible and consider that the education gained from the experiment is perhaps going to be its main product.

CANTRIL: I think experience to date with cancer of the lung from the standpoint of radiation therapy would lead me to believe that, although such an experiment might have some value from the experience gained in designing and using protocols, it would have little value in the evaluation of supervoltage irradiation, primarily because it is a disease in which the energy of the radiation used might bear little relationship to survival.

I think what we accomplish is primarily some improvement in the inflammatory component associated with all carcinomas of the lung, with some increase in the airway through the opening up of the lumen, but the patients in general will die in time measured usually in months, except for those forms of cancer which biologically have a longer survival regardless of what one does with them.

I think we should find some means whereby to conduct experiments. I think we might be more careful in the choice of an objective which will give us a better chance to appraise the efficacy of the agent we are using. In the case of lung cancer, I believe the disease itself mitigates against any clear-cut answer from the standpoint of whether one is using supervoltage or conventional radiation.

KLIGERMAN: Didn't Sandy Watson, at the Hot Springs meeting of the American Radium Society, present a report on carcinoma of the lung treated by cobalt as compared with carcinoma of the lung treated with the betatron? I don't know whether he randomized his material, but, if you remeber, he showed a very lovely graph in which he took the total cumulative survival measured in days or months that the patients lived and he came out with something that looked like this. What he did was to add up the total number of months that all of his patients lived in each series.

DICKSON: The number of patients is the abscissa.

KLIGERMAN: And the ordinate was months. This was 250 kv and then this was 250 kv plus HN_2, and this was cobalt, and this was the betatron series. Strange as it may seem, this was the curve for surgery, except in surgery there were a couple of cases that tailed out like this. He is the only man who has all of these machines in operation, and he felt that there was reason to suspect that the survival was increased using the supervoltage. He felt that HN_2 added to 250 kv perhaps made the survival comparable to supervoltage. He also showed that the areas under each of these curves (betatron, cobalt, 250 kv with HN_2, and surgery) were equal, whereas the area under the curve for 250 kv without HN_2 was less.

STONE: Do you recall whether his 250 kv therapy was done at the same time or using a prior series?

KLIGERMAN: That I don't know, Dr. Stone. I also don't know whether he randomized it. I doubt it, frankly, but at least this is another impression by someone who has had the three instruments that most of us are talking about in operation longer than anyone else, and I think he is a careful observer, too.

QUASTLER: Yesterday I made a proposal for one type of experiment which went over like a lead balloon. I didn't think it was that bad, so I will try it just once more. In connection with lung cancer I tried to say that supervoltage machines can be used to do very precise experiments in radiation therapy, because you can control the dosage and its distribution, the dosage rate, and all the other factors, very accurately. Such studies would probably not yield a greatly increased survival rate, but they might substantially increase our knowledge about what radiation does to cancer.

MOSES: In connection with lung cancer, it would appear that of the many kinds of tumors that can be selected, this one would be least likely to show any substantial change in survival, I presume largely because there are so many metastases, etc. But in addition to the advantages of a sort of shakedown, as it were, in trying to do such a study, I think there is the possibility of some practical advantage from the point of view of future patients. It comes out of the idea that, indeed, there isn't much propriety in thinking of having a single endpoint in this or in any other clinical trial. It may be that survival is the most important endpoint in certain kinds of therapy, but it is never the only one. In the case of lung cancer it may by no means be the most important.

I presume that therapy is given to lung cancer patients because it has certain beneficial effects which have been briefly sketched by Dr. Cantril. It is perfectly sensible to ask then: are these objectives better met by high voltage than by medium voltage? These are going to be more difficult questions to answer, but they will not only have educational value, they may have some practical value in the treatment of cancer in the long run.

KAPLAN: In other words, you can design your experiment to take advantage of a variety of kinds of education.

MOSES: You can look into such things as: is there a better air passage? Reduction in the size of the lesion in the lung? A more comfortable patient? Whatever the objectives of radiotherapy of lung cancer patients are. Apparently it isn't to prolong their lives substantially, but there are other objectives.

NICKSON: It is perhaps worthwhile at this point mentioning the so-called performance index that Dr. Karnofsky has put forward as a means of attempting to quantitate some of these responses during the time between treatment and the death of the patient. What Dr. Karnofsky has done has been to set up a scale from 100 to zero, zero being death and 100 being normal. Ninety percent would be well. Eighty percent would be up and about with some complaint. Fifty percent would be bedridden and at home. Forty percent would be bedridden in the hospital.

This is quasi-objective but it permits a little more regularization of the statement about how the patient feels than just that the patient feels fine or he does not feel fine. We have been using this index to a degree, and I think it can be developed into a useful tool. The important thing is that if we are diligent, criteria can be evolved which are reasonably reproducible and which provide other parameters to evaluate treatment than simply the one endpoint of whether the patient dies.

BOND: I am not sure how much of a consideration this is, but I gather the feeling is that a lesion like this is not likely to produce, shall we say, as dramatic results as other types of lesions. If this experiment turns out to be negative, to what degree will this jeopardize your chance of engendering enthusiasm and support for a longer range experiment on some other type of lesion that might have a better chance of giving a positive result?

KAPLAN: That is a good question and it is hard to estimate. My own reaction would be that if a group that went into such an experiment were fairly realistic about what it might produce and recognized that all they were likely to get out of it was an education on how to do such experiments, so that they could then pick something more fruitful the next time around, --

BOND: Don't the results of this go beyond the group that is doing the experimenting, and don't you have to take into consideration the opinions and thoughts of other groups that would not take this in the spirit in which it is set up? They might tend to downgrade the efficacy of radiation therapy in general to a degree that is unwarranted.

KAPLAN: I don't know whether such a negative study would ever get published, but if it did get published it could be largely as a study of the methodology of conducting such an experiment. The results, it would seem to me, are rather incidental, but maybe this is a biased point of view. I certainly don't want to be in the position of beating the drum for cancer of the lung, but we are not going to learn to walk before we can crawl and this is the main reason for proposing a type of material which, in a sense, you might consider to be expendible.

I would feel worse about our picking something like cancer of the cervix, doing the experiment at a time when we don't know how, and perhaps messing up the experiment in various ways, coming up with fallacious results which could really tend to bias general opinion about the efficacy of radiation therapy.

GARCIA: I think one other point in favor of this is that cancer of the lung is a very frequent tumor; consequently everyone should have sufficient material to study it.

KAPLAN: Yes, and it is also readily available. It is sufficiently discouraging so that most of our colleagues are not too unhappy about letting us have appreciable numbers to study if we have something positive to suggest.

FLETCHER: But not the good cases.

KAPLAN: No, I disagree with you. Our surgeons would at least like to explore all the cases that are not obviously inoperable. But I would have no objection to their doing this and even to their doing a resection where they find it is resectable.

FLETCHER: I think that the treatment of cancer of the lung is surgery.

KAPLAN: There will be an appreciable number of cases which are localized, but which are found to adhere to the aorta, or to go up just a little too high on the carina, etc. These are still localized cases which, though inoperable, are eminently suitable for radiotherapy.

KLIGERMAN: Do you reject treatment of carcinoma of the lung? Don't you treat any cases of carcinoma of the lung?

FLETCHER: Very rarely and I never treat any with supervoltage.

CARPENDER: I think somebody raised the point last night that a cooperative experiment like this will make the selection of cases easier for all of us because we won't be under any pressure to treat patients with supervoltage or otherwise if we have the backing of a large group. We can say that this is an experiment and we are doing it on a cooperative basis, and I think our colleagues and the patients will go along with us much better.

KAPLAN: This is certainly the way it is actually working for the cancer chemotherapy protocol experiments. I have seen at two tumor boards, one at our own hospital and the other at the local Veterans Administration Hospital, the operation of this kind of consideration. Perhaps the tumor board as a whole may not be too enthusiastic about the chemotherapeutic approach that has been proposed, but the question keeps arising: does this case fit into the cooperative protocol under which you fellows are working? If their answer is: "Yes, it does fit in," then the whole group turns out to be willing to let the case be assigned to the study. You can see in operation in such instances a respect for cooperation with a large study being conducted across the country which perhaps would not be forthcoming if this were your own pet idea in your own hospital alone.

KLIGERMAN: We did this with the gynecologic cancer cases six or seven years ago. At that time, the number of cases of carcinoma of the cervix, stage I and stage II, which were being referred to the radiotherapy department, was limited to the cases in which there were medical reasons contraindicating operation and to those patients who frankly rejected operation. In order to get the material and because our gynecologic department is filled with men who do want to find out something, the radiology department went to the gynecologists as a group and said: Look, everybody is doing radical hysterectomies and no one is finding out whether it is worthwhile as compared with radiotherapy. As a result of this a very rigid program has been set up and since that time we have been getting half of all the material. Everybody cooperates. We could not do this without having set up a program.

Another thing is of interest. Dr. Quastler, you were a little pessimistic about the number of cases needed. If you get sidetracked because of that, indeed, you never do have the cases. Granted that it is now six or seven years since the program was started and not all the information that we wanted to find out has appeared in statistically significant fashion. We have at least found out about certain points, and the surgeons now agree that they will not operate on such cases. So that, although our experiment is not complete and there has been no publication, we have certain definite pieces of information that have helped us.

FLETCHER: That is different. You are testing surgery versus radiation. Now we are testing various forms of radiation. The surgeon will not have a direct interest in the answer.

KAPLAN: There is one point that perhaps ought to be clarified here. I do not think it is at all necessary, or even desirable, that every therapeutic institution in the country participate in the same experiment. I think we would actually jeopardize the experiment very seriously if we had too many groups collaborating in any one experiment. Each of us is in a position to know, or at least to guess pretty well, the kind of material that we are likely to be able to make available for a given experiment. Those who can anticipate difficulty with the mammary cancer surgeon would perhaps be best advised to refrain from participating in a specific experiment on breast cancer and to think in terms of the things that they can do and are interested in doing. An ample number of different experiments can be thought up, because any one of us can name innumerable problems in the field of radiotherapy for which there are no universally acceptable solutions. We have talked about problems of total dose, about problems of fractionation, about problems of scheduling of treatments. Not only the number of fractions, but their spacing in time. None of these things are known with any certainty.

Some time back, while sitting on an airplane with nothing better to do, I did some thinking about the necessary steps in setting up a cooperative experiment. I don't know whether this will be helpful or not. We have been over much of this ground. It seemed to me that some of the considerations went as follows:

First of all, regarding the type of clinical problem to be selected for

study, one consideration obviously has to be the frequency of the particular form of cancer. There is very little scientific reality in a study of the efficacy of radiotherapy in sarcoma of the ligament of Treitz because it just does not happen very often. So frequency is obviously an important consideration.

Second, the point that has just been discussed, the competition for such material from other sources, the surgeon, the gynecologist, etc., will determine the number of cases actually available.

Third, the radiation response ordinarily to be expected. I am not sure that there is any point in making a study, let's say, of osteogenic sarcoma.

Fourth, the kinds of endpoints that a particular tumor would lend itself to studying.

The next item would be the number of participating institutions. Here one would have to think about keeping any one experiment small, initially. There can be as many experiments as there are institutions wishing to do one experiment or another.

One would have to consider the caliber of the radiotherapist, the physicist, and possibly the radiobiologist, and certainly of the statistician, available in each of these institutions.

One would have to consider for a given experiment whether it necessarily involved supervoltage or not. If the experiment implicitly involved the use of supervoltage therapy then only institutions having such equipment could participate.

There has been some debate as to whether such experiments should be designed regionally with institutions that are more or less neighbors trying to work together, or whether it should be national, with the institutions distributed more widely. In such a situation where should the statistical headquarters be centralized? If we are going to go in for such things on a rather large scale, there might be some virtue in thinking about an agency, similar to the National Cancer Chemotherapy Service Center at Bethesda, merely as a clearing house. Perhaps in the early stages of this venture there is no indication for such a center, but I can see some virtues in it later on. In any case, anyone trying to do such an experiment would have to decide who is going to handle the central statistical responsibilities.

The methodology is my next heading. I think the participants would have to get together and agree on the preliminary workup of the patient. What procedures are going to be done? What type of information will be made available about the clinical entity that is under study? Such things as proof of diagnosis, evidence concerning extent of the disease, a metastatic survey, any special studies that are to be conducted on that particular kind of cancer. Agreement on classification or staging, as Dr. Robbins brought out earlier, is really crucial. Even if one employed a classification that is not universally acceptable, it seems to me that there is validity in picking a classification which has a working base that is acceptable to the group.

It would be best under such circumstances, if there is no international classification, if the group were to describe or specify its classification with a good deal of precision. Then later on, if others don't agree with such a classification and yet want to interpret what has been done, they can transpose the classification stages in some other way.

One would then have to spell out very explicitly what kind of case out of this pool is going to be considered for study.

Next we come to something very difficult, the exact method of radiotherapy that is to be employed. We have not discussed this in detail but I think it would be essential to agree on one technique for the purposes of the experiment.

In my opinion, this would have to be a rather detailed plan, much more so than in the cancer chemotherapy protocols. Dosage, volume distribution, the methods of dosimetry, the treatment time, the numbers of treatment per week, and so on, all would have to be specified rather carefully. Any other methods of treatment which were going to be employed in a particular experiment, such as oxygen, chemotherapy, and so on, would also have to be specified very carefully.

Then I think the group would have to agree on the evaluation of the initial response. What happens in the first few weeks with regard to tumor regression, improvement of the clinical status of the patient, and so on. What observations will be made about this, how will they be made, and how will they be graded?

I think some consideration would be necessary about complications and early and late sequelae, and the group would have to agree on what they are going to look for, how they are going to look for it, and how they are going to tabulate it.

The group would have to find out from the statisticians what they do about their randomization when treatment has been interrupted because of some complication or when death terminates the treatment of a particular case prematurely. This is a very tough kind of problem which I know is calculated to throw statisticians into a state of despair. But they have to be brave fellows, too, and face up to these rather natural difficulties. I think there are at least some crude solutions to these problems which they can give us.

I think there has to be agreement within limits on supportive treatment. Is there to be free use of transfusions, antibiotics, and so on? What about bed rest and hospitalization? This is used freely in some hospitals and not in others. There must be some general understanding about the extent to which patients will be hospitalized.

This may all sound trite to you, but I think it is desirable to look at these details as an indication of the kind of job that has to be done before the

experiment can be initiated. I have had occasion to read some of the chemotherapy protocols and I have seen the work that they have put into their experiments.

There would then have to be agreement about the length of follow up, the statistical analysis that is going to be applied, the handling of cases "lost" to follow up, and so on.

Then a rather mundane matter: how is this work going to be published, if you have six people participating in the study, whose name comes first? I may be anticipating difficulties, but I think the statistical solution is obviously the best one: you randomize.

Finally, I think the group would have to face up to the problem of intercommunication and liaison.

FLETCHER: It would be a test of enthusiasm.

KAPLAN: I will finish with just one other point, one that Mr. Cornfield brought up earlier. I think at this point one can ask the statisticians for guidance on the approximate total number of cases needed to establish significant differences at the significance level agreed on, and on the basis of the best estimate that the group can make of the probable observed difference in endpoints.

One thing that perhaps you didn't emphasize enough is that it isn't the statistician's job to decide whether this is likely to be a 10 percent difference. The therapists themselves must supply this information, as the best estimate they can make, to the statisticians. If they are overly enthusiastic they will bias the chances for the success of the experiment because they will get too few cases into the design of the experiment; conversely, if they are overly pessimistic they are making life much more difficult for themselves.

KLIGERMAN: You can decide that question later.

CORNFIELD: No, this has to be specified in advance. I should like to restate that problem just slightly. This is not really a question of anybody's estimate as to what the difference will be. That is for the experiment to determine. This is a judgment on how big a difference you are interested in detecting.

BOND: Why must this be specified in advance?

CORNFIELD: Suppose you don't. There are several answers. One is that the mathematics do not work unless this parameter is put into it. But let me give you a more convincing answer than that. That is, suppose you make a judgment without reference to the size of the difference you are interested in detecting. You say that you will take 200 cases and you go ahead and do your experiment. You come out with a set of results and you test them for significance and you conclude that there is no significant difference. What does this really mean? It does not mean that there isn't any

difference. It means that if there is a difference it is less than some magnitude.

Now what is this magnitude? Suppose the magnitude is 20 percentage points. Suppose at this time you say: this isn't really a decisive experiment because all it has told me is that the difference is somewhere between zero and 20 percentage points. At that point you have to start all over again if you are interested in knowing whether the difference is statistically significant.

BOND: Why can't you specify narrow limits and then stop if it becomes apparent that you don't need them?

KLIGERMAN: That is what I meant by saying that it was possible to test at each time period when the number of cases available can be tested for a certain percentage.

CORNFIELD: To give a really clear answer I would have to make a long talk, but let me make a short one. There are methods for doing this. They are called sequential analysis methods. You don't decide your sample size in advance. There is a very clear article in the recent issue of the Journal of Medicine explaining sequential trials. I would say that in principle these methods can be employed, but I myself am a little uneasy about employing them in a clinical trial. I don't think we have enough experience. That is point number one.

Second, if you simply say: after I finish treating every case I am going to do a test of significance and stop when I have a significant difference at the 5 percent level, then you are playing a game that is really illegal. The mathematics of our significance tests say that you are going to keep on going until you have treated the prespecified number of cases and then you are going to make one statistical test. If you make a test at every point, you have a much bigger chance of coming to an erroneous conclusion than the .05 that we put on the board.

MOSES: I will give you an example. Let's take a coin. We are all convinced that this coin is going to come up heads 50 percent of the time. But actually you are going to toss and after each toss you are going to test the hypothesis that this is a biased coin. You are going to keep on doing it until you come to the conclusion that the coin is biased. We can demonstrate that you can eventually conclude that this coin is biased no matter what level of significance you are testing.

BOND: Will you say that again?

MOSES: The whole thing? We can demonstrate that you will eventually conclude that the coin is biased.

FLETCHER: That is what we want.

MOSES: You say: I am going to flip it until it is significantly biased at the 5 percent level. It can be proved, if it is a fifty-fifty coin--you may be

an old man by then, but eventually you are going to get a result significant at the 5 percent level or whatever level you set out for. You say that the coin is crooked, but it isn't the coin that is crooked; it is the method of testing.

FRIEDMAN: Everybody here has played the game of the first two out of three. You find you have lost and you say to the other fellow, "Let's play three out of five." It is the same thing?

CORNFIELD: Yes.

MOSES: May I make a remark on sequential analysis? This discussion grew out of your point of specifying in advance a possible difference between two treatments. You spoke of it as an estimated difference, and Mr. Cornfield said what I would have said, that it is not really somebody's guess as to what the difference is going to be. It is the specification of a difference which would be big enough to be practically significant.

KLIGERMAN: I was going to ask that question.

MOSES: Now in the case of sequential analysis you may not decide the sample size in advance. Instead you decide on a rule, and the rule does not happen to be to keep going until you get a result that is significant at the 5 percent level. There is a different kind of rule and it avoids this difficulty. In order to specify that rule you don't specify the sample size, but you still have to specify a practically significant difference. You don't get rid of the problem that way. In order to say what the rule is you are still presented with specifying that either the treatments are the same or they differ by blank percentage points and once that has been specified and these two numbers, alpha and beta, which Mr. Cornfield spoke of earlier, have been chosen, then instead of getting a fixed sample size you get a fixed rule with a variable sample size. It ordinarily requires smaller samples, and it ordinarily is a much more complicated job to keep track of it. Also it would not be good in certain instances. If you are taking survival as an endpoint, you are not particularly interested in taking the observations one at a time if there is five-year survival, because the study will never end in finite time, so that there are no medical contexts in which you can really use sequential analysis.

KLIGERMAN: I want to ask this question just so it is definitely in the record. Is a 10 percent difference the difference which the radiotherapists would consider to be a significant improvement of one method over the other or is it some other figure?

BOND: Ten percent of what?

KLIGERMAN: Ten percentage points.

MOSES: Is that 10 more cures per 100 patients presented?

KLIGERMAN: I am not suggesting a specific figure. I want an expression of opinion of what we, as radiotherapists, will accept as clearly demonstrating that one method of treatment is better than another.

KAPLAN: I think you have to make it a little more explicit. Again to take cancer of the lung as one situation, if you can boost the cure rate of cancer of the lung from 5 to 15 percent, which would be what you are talking about, and if you apply these percentages to the actual number of deaths--I am not sure just what they are at the moment but I would guess of the order of 30,000 deaths a year in the United States--this is a salvage of 3,000 people every year--I do not think there is anyone in the room who would not think that is very significant.

LAMPE: What you are saying involves raising the radiotherapeutic cure rate from zero to 15 percent.

KAPLAN: Even if you raise the radiotherapeutic cure rate from zero to 10 percent and the surgeons go on curing the 5 percent that they do cure, then the total cure rate is 15 percent.

LAMPE: What I am getting at is I think to contemplate increasing the therapeutic rate from zero to 10 percent is completely unrealistic.

KAPLAN: We are not talking about realism now. We are talking about what the group would consider an important contribution.

LAMPE: There is no question about that.

KLIGERMAN: How about a real 10 percent improvement in results in any disease over what we have now in that disease?

SCOTT: Dr. Kaplan, may I make just a few remarks? I don't know what would be the significant figure we are looking for, but it seems to me that time is beginning to run out on your committee and this meeting. It seems to me we ought to get together and get some constructive things decided upon, so that future meetings can be arranged and certain processes can be put under way.

First, there is interest here in having a cooperative research program. Cooperative research programs are in fashion now and are productive. I haven't seen one that has not created a great deal more interest, that has not resulted in more benefit, that has not gotten more people interested in the program, and that has not been productive.

This procedure has been applied in the Veterans Administration and is perhaps the most productive method they have evolved. It is being expanded into all of their educational and research programs, and has been approved by their advisory committees composed of topnotch men in the country.

So I think, first, that this group should decide whether it wants to go into a cooperative program. It is very obvious that no one of us has enough cases to make a decision on anything. Second, any of these cooperative programs have to be done on a voluntary basis. The participants have to be volunteers.

If these decisions are made, the group then has to decide: can we evolve

a short-range program that will give us some significant results in cancer of the lung? What can we do about the long-range program that Dr. Fletcher outlined? This might include the treatment of lesions of the pharynx that can be inspected and from which you can get biopsies, which you can stage and observe and record the various endpoints that the group decides on.

If you want to do this then it seems to me that you are ready to set up a center, or something corresponding to a registry in Washington, under the auspices of the Radiation Study Section or Bob Andrews' group, or perhaps the National Research Council.

Then you are ready to form a couple of committees. One to go into the setting up of a protocol, standards of radiation therapy and the things you mentioned in your talk about cancer of the lung. Have that committee set up those criteria and procedures, and write them all out. You cannot get anything done until they are down on paper. The same thing would apply to Dr. Fletcher's recommendation for a study on the pharynx cancers.

These two committees should then come back and give you reports--you can all have them beforehand--and the whole group could then decide on a program. I think then you can get on with this program.

We have discussed all of these things and we are going back into a lot of details that can be worked out by these committees in consultation with Mr. Cornfield and other statisticians. They can come back to the group with a constructive written program that can be sent out to the members prior to the next meeting.

KAPLAN: I think that is very helpful, and it condenses to practical terms where we stand at this point.

I wonder whether this might not be the time to get the sentiments of this group, without committing any of you individually as to your participation. First of all, do you think it would be a good idea to go ahead with attempts to plan such experiments. It would in no way obligate any of you, even those who raise your hands in support of such a program, to get involved in it when it gets started, if it ever does. Could I just see a show of hands of those who, on the strength of what we have talked about, feel that there is a need for trying to design cooperative experiments on some aspect or other of the field of radiotherapy? (Practically unanimous.) There seems to be a substantial enough majority so that perhaps I need not ask if there is anyone who is strongly opposed. However, if anyone wishes to record his opposition we will be glad to hear his remarks.

ZIRKLE: I was not sure who was supposed to vote.

KAPLAN: I think we will simply record this as a strongly affirmative vote.

SCOTT: I should like to make one other observation here. I think this meeting has been extremely interesting and satisfactory because radiation

therapy by itself has never had an organized basic science below it. Internal medicine has. Pediatrics has. All the other fields have had, but we never have had and it is the radiobiologists who are going to be our basic science group. It is from their work that is going to spring the stimulus, the inspiration, the niceties, the understanding of cell life and cell reaction that we are going to utilize clinically. So I think that they should be included in the formulation of our protocols. I think the more we can made them a part of us the stronger the whole picture is going to be. I should like to see them vote.

KAPLAN: The only reservation I have about that speech is that I didn't make it myself.

CHAMBERLAIN: I think this is an extremely important point, and that we ought to set up the group so that it has a strong representation of radiobiologists, radiological physicists, and statisticians.

SCOTT: That is what I meant. They have a full vote, a full voice. They are as much a part of this group as anybody.

KAPLAN: I think that would be a very salutary thing. As far as the mechanics are concerned, perhaps the next best step would be something like this. We are really confronted with two possible kinds of conferences. The one we are talking about right now is a working conference or perhaps a series of conferences involving a very small group. There could actually be several working conferences, depending upon the number of experiments that one wanted to entertain. But I think each working group should concern itself with only one explicit experiment. My tentative suggestion would be that we take a little time to deliberate on the kinds of experiments we are really interested in, taking into consideration the kinds of material we have on our own home grounds, the kinds of cases that we could supply to a study, the facilities that we have, and the kinds of interests that we have.

Then I should like to suggest that each of the therapists here who is interested in participating in a working conference to design an experiment write a letter to indicate his interest and take the trouble to outline the kinds of experiments that are generally of interest to him.

SCOTT: May I comment on that? I think you have the group right here. When Dr. Fletcher gets home he is not going to sit down and write all these experiments out. He has other things to do. You know pretty well right here the two kinds of things you are going to investigate. Go ahead and form your committees for those two fields. Let them have their meetings and evolve a plan right off. I think you are wasting time if you wait for everybody here to write a letter about his idea of an experiment.

KAPLAN: I was not asking anybody to design an experiment. I was simply thinking that some people are going to be interested, let's say, in an experiment on pharynx cancer, others in lung cancer, but still others may come up with notions about a high oxygen experiment having to do with neither of these.

SCOTT: Don't you think if we are going to have a cooperative experiment, we ought to decide on what fields now and then have volunteers go into those fields, instead of sending in 10 or 12 different experiments or ideas?

KAPLAN: I should like to hear from the group about this. One reservation that I have is that we don't have by any means all the first-class radiotherapists encompassed within this room. There are some very good people who are not here for reasons beyond our control. They either were unable to come or there isn't room enough to fit them in. If we start out by designating people here to get this thing off to a start, we are almost by the very nature of things excluding a number of our colleagues who are in a position to make real contributions to the design of such experiments.

Second, if I were to take the prerogative here of appointing committees this perhaps might turn out to be embarrassing to the Public Health Service, since a few of us here are on the Radiation Study Section, which is one of the sponsors of this conference. Perhaps it would be better in this sense to have people write in and say, "I would like to participate in such a thing if you will organize it."

SCOTT: If you can tell them what to write in about.

KAPLAN: Dr. Curtis, I wonder if you would like to comment on the method by which we might proceed from here?

CURTIS: I don't have any definite ideas on it. I think the Radiation Study Section is pretty well set up at least to see this group effort through its formative stages, by providing a clearing house, the necessary funds, and secretarial work, to get the program going. I think that the most convenient way to handle it is through the Radiation Study Section, since the mechanics for doing so are all there and ready to be used. I am sure that whatever the group in Washington can do to expedite it they will do.

CANTRIL: I should like to make one suggestion, since I assume that no grants are going to be dispensed here, that if possible we invite some of our Canadian colleagues to participate if they so wish.

CURTIS: There is no problem here that I know of. I think that is a fine suggestion.

KAPLAN: I think there is one thing that perhaps I should make explicitly clear. Obviously, I am going to be interested in participating in such a thing as well as perhaps all the rest of you. This conference is an exploratory conference and it commits nobody, including the Public Health Service. If working conferences do get going and groups do undertake to design experiments, it should be clear to them that even after they have done so, there is no guarantee that anybody is going to support the experiments. The experiments must be good enough to merit Public Health Service support, on review by the National Cancer Advisory Council. I would guess that if the experiments are carefully designed and thoughtful and realistic there will be no problem in support. But I would not like anyone to get the idea by

indirection that the mere fact that some of the members of the Radiation Study Section have been instrumental in organizing such a conference as this means that any plan that emerges is automatically going to be pushed through.

There is clearly a need for a good study, but there have been decades of studies that have proven little or nothing. So that the Cancer Council of necessity will look pretty hard at the details of any plan that comes before it.

In actuality, with the kind of statistical help that we are fortunate enough to have here, and with the years of experience that some of the therapists in the group have had, we should not have too much difficulty designing some realistic experiments. So that while I would like to take advantage of the flush of enthusiasm of the group now, I really think that perhaps there would be some disadvantage in trying to assign people to committees right now.

I hate to lose any more time, but I wonder whether we could not ask those who really want to get this program going to write in and say so. They could write, I think, to Dr. Himmelsbach, the Executive Secretary of the Radiation Study Section, National Institutes of Health, in Bethesda.

HIMMELSBACH: The address is complicated enough, just like our experiments, without making it any worse:

Dr. C. K. Himmelsbach, Executive Secretary
Radiation Study Section
Division of Research Grants
National Institutes of Health
Bethesda, Maryland

MEADER: I am very glad that you said what you did about the interest of the National Institutes of Health, which includes the Radiation Study Section and the National Cancer Institute and other institutes.

I think there is one other point that is worth mentioning. It came up in connection with our Canadian colleagues and it could be true for others outside the country if that seems to be a feasible thing. That is, there is nothing that prevents cooperation on an intellectual level regardless of the source of the funds for support. The most important thing is cooperation on the intellectual level and a common approach. There are various sources of funds, local and national and international, which may be interested in financing such a study.

It has been our experience with some cooperative groups that there are individual members of the groups who need practically no funds outside of their own local resources to participate. There is also the experience that one group may have a particular relationship with some fund-granting agency which they would like to maintain. I do not believe there is any representative here from the American Cancer Society or some of the other fund-granting agencies, but I think it is fair to say that all of these agencies that are concerned with cancer have an interest in improved radiation therapy. Canadian representatives, or representatives from other nations, who might want

to participate in such a study might have their own local funds. Also there is nothing in the regulations of the National Institutes of Health that would prevent aiding such groups if it were necessary.

I think it is important to emphasize, as you did, that the interest of the fund-granting agencies in helping to get the initial development of the program under way does not and cannot commit the agencies to subsequent support prior to the presentation of a proposal that can stand on its own merits. We have had some experiences of a kind that can be potentially embarrassing, if the impression is given that the agency has committed itself in advance to support something which on subsequent review it finds that it cannot support either because the scientific merit is not great enough or because funds are not available.

I think in general, as far as the National Institutes of Health are concerned, particularly the Cancer Institute, if there is demonstrated need for investigation by competent people, funds can be found. I would like to end with that little note of optimism.

SCOTT: I think at your next meeting it would be very desirable to have a representative from the Veterans Administration, the Navy, the Army, and the Public Health Service. These groups have a controlled patient population. I am sure that they would be very keenly interested in participating in such experiments. They have certain centers where they have the facilities to participate. Having them come to your early organizational meetings would stimulate a continuing interest on their part.

KAPLAN: I am personally very gratified with the sentiments that have been expressed today. I do not wish to take undue advantage of your current enthusiasm to the extent of formalizing a series of working committees, but I do think that perhaps a partial approach to this could be made. It is obvious that different kinds of experiments are involved here. We talked about oxygen. We talked about supervoltage. We talked about lung cancer, we have talked about cancer of the pharynx, and we have talked about cancer of the cervix. It may be that several different protocols will be developed more or less concurrently.

It would help any one of these initial working groups if there were one person for each of them who would be willing really to sit down and do some spade work in advance, to draw up the physical, biological, clinical, and statistical considerations in the form of a rather detailed rough draft of a preliminary protocol. If one person for each of these experiments could be found who was willing to volunteer to put something down on paper that could be circulated to the group that had expressed itself as being interested in the particular kind of experiment, it would seem to me that this would save time.

Now just to jell something at this point. I should like to ask whether someone here is not interested in doing this for the high oxygen situation. I have a candidate in mind, but I don't know if he is going to volunteer. Dr. Wood, can you be persuaded to volunteer?

WOOD: I am interested in it, but I think it should be designed in collaboration with a therapist. I will be interested in talking about it.

KAPLAN: If you want, you could do it collaboratively. This is a problem which reaches into physics and biophysics and you have the necessary background on that side which we as therapists do not have.

WOOD: Why don't we talk to Dr. Chamberlain? Are you interested in this field?

CHAMBERLAIN: Yes, the only thing is I won't personally be able to take part in such an experiment for at least a year if the design involves supervoltage, but as far as thinking about it is concerned, I will be glad to undertake it. But I would think again, if we are going to take up each one of the design concepts as Dr. Kaplan did--your outline for this, incidentally, covers about the same material as my own--we have to have a little larger than a two-man nucleus to work on it. We at least have to have contact with the statisticians on the design, too.

KAPLAN: My thought was that the statisticians would be at the working conference and they would pick up the ball at that point, but maybe the group does not feel this way. I was not thinking of a completely finished protocol by any means.

CHAMBERLAIN: But the major things that you would take up are choice of material and methodology. I mean, policy is already decided, and when you say that we are going to try to work up a protocol on a high oxygen treatment, in order just to derive a preliminary protocol we still have to have a statistical design.

WOOD: Since we are next door to each other, so to speak, it may be that someone on the campus can aid us in the preliminary design of the experiment.

CHAMBERLAIN: Or possibly one of the statisticians here might come and give us advice for one day or something like that.

KAPLAN: Both Mr. Cornfield and Dr. Moses have expressed their real interest in continuing to work on this kind of problem with us, which I think is extremely encouraging.

CHAMBERLAIN: I would say one more thing; that is, I think this is a more difficult one actually than, say, either lung cancer or the hypopharynx if those are the three we are going to think of.

KAPLAN: Let me make it clear that I don't wish to nail this down to any specified number. I think there should be as many experiments as there are interested people willing to conduct such experiments.

CHAMBERLAIN: Ought not, however, consideration be given to keeping the first workups down to not over three, largely to give us experience in

design? I think if we start out to try to write up protocols for ten different experiments we are going to get bogged down.

NICKSON: I was going to suggest that rather than picking on a given lesion at this time, two other groups in addition to the oxygen group might be set up to consider what might be called the short-range and the long-range evaluation program. We could leave it to these working groups to make up their own minds as to which lesion they wish to put before the full committee at its next meeting.

JOHNS: I just wonder whether you might consider variations on the procedure of high pressure oxygen. Three atmospheres, as any of you who have read the article know, is a very excellent procedure. While it may not fall in Dr. Fletcher's classification of practicality, it is a very interesting procedure and perhaps should be tackled by a very small number of places. Another approach is really to try to get some results on patients breathing pure oxygen at one atmospheric pressure. I know this is being done in a few places, but they are not likely to have enough patients or will not have things under good enough statistical control to arrive at a conclusion. Nonetheless, this is an experiment that could be done relatively easily. It could be arranged so that the therapist does not even know whether the patient is breathing oxygen or air out of a cylinder.

WOOD: To my mind, at least, we have overplayed this concept of practicality, because there are things that may not be practical today that will be practical ten years from now. I can certainly appreciate that from the clinician's point of view you want something that is easy, but I think it is the responsibility of any study group to look ahead five, ten, or twenty years, because as I understand the situation from what has gone on here, not too much has been advanced in the last 40 years in radiation therapy. Am I wrong?

KLIGERMAN: Yes, you are wrong.

FLETCHER: The concept of practicality was mentioned with reference to megavoltage equipment which is decidedly much more expensive. One still has to be practical.

WOOD: There are other ways to get around the disadvantages of high oxygen pressure. As Henry mentioned this morning, it may be that one can increase the local blood flow rate in the particular region that is under therapy. There are many things that can be investigated. So I don't think that we should close our minds as to the technique that is involved. This is something that we have to study in great detail. Also I would certainly feel that in this kind of experimental program, we would look forward to accomplishing something over the next ten years rather than something we can use tomorrow.

CARPENDER: Why not get Lanier's help on this, since he is already doing it? I am not suggesting that he draft it, but that he at least tell us what he is doing and give some help. Also, is it worthwhile even to discuss

any of the details here? Isn't it up to us to get somebody started and to leave the details out at this time?

KAPLAN: Yes, I think there is a temptation to get into details here which we should resist. Dr. Nickson's suggestion really strikes me as a very good one. What we are really confronted with are three or four different kinds of questions that are not necessarily separate in the final development of the experiments. That is, the high oxygen question can later be considered in connection with the question of lung cancer or any other type of tumor. Perhaps we need somebody to set forth the pros and cons of the oxygen experiment without regard to what kind of cancer it is going to be tried on, going into the physiological considerations, problems of measurement, problems of anesthesia, and so on. Then someone else is needed to really cudgel his brain about what Dr. Nickson has referred to as the short-term kind of study, which for the most part would be related to rather hopeless material but where short-term observations might help us at least get educated. Maybe there are better kinds of such material than lung cancer, in which case it would be up to anybody volunteering to handle that topic to document the case for doing whatever is to be done. Still another small group of volunteers may wish to consider practical approaches to clinical material in which long-term follow-up will be essential. Finally, there may or there may not be some virtue in having Dr. Kohn, for example, give further consideration to practical approaches to the question of fractionation and tumor recovery rates and studies along that line.

NICKSON: I have just talked this over with Dr. Kligerman and we would be willing to attempt to get something down on paper on short-term evaluation. We would, I think, by the same token be most grateful for any suggestions that anyone has, in an attempt to reduce to writing something for consideration by a working group. We will be willing to do that.

KAPLAN: That is fine. We will consider that Drs. Nickson and Kligerman have just volunteered to develop a preliminary protocol on the short-term type of study, and that Drs. Wood, Robbins and Chamberlain, the Philadelphia contingent, are the high oxygen group.

CHAMBERLAIN: Incidentally, one reason I think I would be willing to attempt this is that there is a very active, functioning group of physiologists in Philadelphia. I think that we have in people like Chris Lambertson probably as good people on high oxygen toxicity as there are in the United States. We could try to draw on some of these people for help.

Who has seen the work in England specifically? Are you the only one, Dr. Nickson?

KAPLAN: I have pictures of it.

FLETCHER: I am going to see it this summer.

CHAMBERLAIN: If we are really going to try to do this, we would like to have as much information about the difficulties in these things as we can.

KLIGERMAN: We are in the process of designing a tank right now. I think the tank design is very important. The one that Churchill-Davidson used was really a makeshift tank.

KAPLAN: I think perhaps it would be best, since these are details, if each of you who possesses specialized knowledge about these matters would take it upon yourselves to communicate with the subcommittees that we are now delegating to do these jobs. It will help them enormously, and I will simply leave it to you to contact them. If you know of people who are not at this meeting who have specialized knowledge along these lines by all means bring this to their attention also.

Now we get to the question of these longer range studies, and I have been wondering whether Dr. Garcia can be persuaded to do some volunteering. You won't have to do it alone. It seems to me that this is the kind of problem where people of considerable clinical experience are needed. I should like to see some of the more experienced therapists in the room try to come to grips with this question in a constructive way. We have all been over the difficulties and now I should like to see the experienced group try to tackle the possibilities. Dr. Garcia, would you take the responsibility of picking your colleagues who have this kind of experience?

GARCIA: I will be glad to, but I should like to have the help of as many as possible.

KAPLAN: Why don't we leave it at this: you will take the responsibility for getting this started and I will leave it to you to name your own subcommittee. This need not be one proposal for that matter. It might be well to consider alternatives of various kinds.

Along this line, Dr. Stone earlier asked a question which I think he should ask on the record.

STONE: There is one thing that I think can come out of this meeting, if I judge the spirit of the meeting well. That is that we have already reached a decision on supervoltage to a certain extent. There are a large number of problems, as our statistician friends pointed out. You can solve any of these things. One that is apparently not solved is how many more people can you salvage over a five- or ten-year period with supervoltage as against the other. That is the problem that we have facing us.

The other problem that I would like to put up is that we have already solved the problem of the fringe benefits, as it were, of supervoltage. I have heard nobody say anything against supervoltage as a means of treatment except maybe that it is expensive, but nearly everybody has indicated that he would not like to give up his supervoltage unit.

What I should like--and this is the question that I proposed to Dr. Kaplan a while back--is how many here who have supervoltage machines would now be willing to give them up and go back to using 250 kv machines exclusively.

KAPLAN: That isn't the question that you asked me a while back.

KLIGERMAN: But it is a good question.

KAPLAN: All right, if you want a series of questions like this let's have them, but I hope you will get around to the other question sooner or later. How many here would abandon their supervoltage machines? (None.)

STONE: I think that is one answer to the problem we came here for, that regardless of whether you are saving any more people or not, you want to use supervoltage. The next problem which becomes a little more difficult is if we want to decide the problem of whether we are going to save more people over a prolonged period of time, it has become clear here, if it was not before, that you cannot take past statistics with different techniques, even though you may say that you are trying to get the best out of the machine now. The best you can get out of a machine is better, or it should be better, than they got out of it ten years ago when the statistics were gathered. How many here would be willing to take patients with cancer of the base of the tongue in all stages and put half of them on the supervoltage machine and treat the other half on 250?

GARCIA: Some of us don't have supervoltage.

STONE: Providing you have it.

KAPLAN: Let's assume that we all have both. Well, about five.

FLETCHER: If you have supervoltage, you have seen the difference.

GARCIA: Yes, but I would be willing to do it.

FLETCHER: Five years ago I would have been willing to do it, but after three years of supervoltage I am not.

STONE: Dr. Kaplan put another question to me which I think is probably something that might meet with a little more unanimity shall we say. That is, maybe you would not be willing to take the early lesions, but perhaps you would take the advanced lesions. Most of us know that we have about a 5 percent chance of doing something for them in the region about the base of the tongue. Of course, when they get to be big you are not too sure where they started. How many would be willing to take those lesions and treat some of them on 250 kv and some on 1,000 kv?

It is the same thing as the lung cancer. Are you going to prolong the lives of some? In other words, what you are trying to prove now is whether you can prolong the lives or increase the five-year survivals of some types of cancer where you have some chance of doing something.

FLETCHER: I have the answer there. A small group but still about 24 cases in each. I used to get 25 percent freedom--I mean lack of disease at the primary site with 250 kv. I get 80 percent now with Co^{60}. Why do you want me to go back?

ROBBINS: Dr. Stone, may I ask your question somewhat differently. Does anybody believe that supervoltage would be inferior to 250 kv in any of these lesions of the tongue?

STONE: That is essentially what we are getting at. Really I don't know what we are getting at in this whole problem unless it is to decide that supervoltage is better than 250 kv. Let's look at this from the point of view of the country as a whole. The 250 kv machine costs way less than a million-volt machine. Economically any place can afford a 250 kv machine. They cannot always afford the proper brains to run it, but that is beside the point. If they can have these 250 kv machines and if there is no advantage in the big machine, then why should we put any more of them around the country? Let's say that we are the Public Health Service or we are the Donner Foundation and we want to do the best we can for cancer. Are we doing the best we can by putting a lot of million-volt machines around or should we go ahead with more 250 kv machines? I think it is the consensus of this group of people with clinical experience that they would not give up their million-volt machines; therefore, they must think that they are superior in some way. So we have one answer to our problem, although not on a statistical basis.

The last problem that I should like to present is that of megavoltage. I was hoping that this group would take up a little more thoroughly the problem of machines beyond the two million-volt range; that is, the six million-volt linear accelerator, the 22-million volt betatron, and in my case the 70-million-volt synchrotron. There we don't have any additional fringe benefits. You have them all when you reach the million-volt range. Now it is a question of whether you can do anything more by a little better physical distribution of the dosage. That does not save the patient from getting nausea. That does not limit the field much more than it did before. That does not give less skin reaction. I don't know how we are going to reach a decision on that. It might be easier for those who have both supervoltage and megavoltage apparatus to decide to put certain groups of cases on one or the other type of machines. That does not concern too many in this group.

GARCIA: I think there is a more important problem. I think there is very little question about the indirect benefits to be obtained from the million-volt range, up to about 3 million volts. I think the question that is still in doubt is whether going beyond that energy range is really going to make some additional benefits materialize. I think that is where doubt exists, and I think that it is economically very important.

BOND: Doesn't this come into the picture, apropos of Dr. Stone's comment as to whether to put million-volt or 250 kv machines about the country? How many people are there in the country who could benefit by any type of X-ray therapy who do not now have access to it?

KAPLAN: This is difficult to answer, because the qualifications of the people administering the therapy necessarily comes into the equation and that is pretty hard to measure.

QUASTLER: In a roundabout way, Dr. Stone, the experience is that the

therapy machine is quite a valuable gadget because it places the hospitals that have money for a 250 kv machine but not the money to run it properly in a very dangerous place.

KAPLAN: Implicit in that, of course, is another problem that we haven't even touched upon today which is terribly important. Maybe it ought to come up at another conference. The dissipation of therapy material is an enormous problem. It has been encountered in other countries, too. It would seem to me that any group that wishes to devote itself seriously to the major problems confronting radiotherapy has to tackle this one, too.

There is the question of the need for additional conferences of this general, broad nature to be held perhaps once a year for the next three or four years. Does this group, on the strength of what has been accomplished at this conference, feel that this is a worthwhile way to go about the exploration of other questions? I should like to hear from you because this may determine the attitude of the Radiation Study Section as to the need for further conferences.

FLETCHER: I think it would serve a useful purpose for the clinician. I am not sure about the radiobiologists. They may be wasting their time. I have enjoyed listening even though I didn't understand all of it. But all of our ramblings since yesterday noon have been a waste of time for them.

STONE: You want them here.

FLETCHER: I said it was good for me.

BOND: This has been very profitable for me.

LAMPE: I have enjoyed the opportunity to study human material for about 20 years and I am not ready to give it up now. I think we can hold some good clinical conferences.

KAPLAN: I would say if there is going to be a better rapport between the clinical radiotherapists and what Dr. Scott described as the basic science of radiotherapy, namely radiobiology, it is important that we bring both groups into the same room once in a while. Those of you who are therapists have been exposed to a fair amount of radiobiology at this time. You sat through it with fortitude and courage, and perhaps it would not be too much to ask the radiobiologists at some future time to sit through some of the clinical problems. They would come to understand what the therapist is up against and I think this would help them in making a contribution to the solution of some of these difficulties. It is important for us to learn to talk the same language and the only way they are going to learn our problems is to sit patiently and listen to our ramblings for just as long as we sometimes sit and listen about sea urchins.

ZIRKLE: I should like to remark that although I personally haven't said anything for the last two days I have been awake and I have been listening.

KAPLAN: I take it that there is agreement that further annual conferences would be of interest to most of this group.

FLETCHER: Very much of interest.

KAPLAN: And I take it that in the very near future working conferences should be gotten under way to tackle the detailed problems of setting up some of these experiments. We have already named some subcommittees.

MOSES: Probably the question is unnecessary but I am not sure, so I will raise it and be told that it is unnecessary. That is this matter of comparing of high voltage and very high voltage therapy. Would this naturally be a part of the thing to which Dr. Garcia would address his attention, because if not, it seems to me it is a topic that probably does deserve a separate study group.

GARCIA: I see no reason a separate group could not study that.

KAPLAN: Do I see a volunteer? Dr. Fletcher has had about as much experience with a comparison of energies in the range of 1 to 3 million versus higher energies as anyone else. Would you like to undertake to put something down on paper?

FLETCHER: Yes.

KAPLAN: You are free to draw on the help of anybody else you want to call on. You know who the people are who are interested in this field. I would say in this connection that there is no reason Dr. Watson could not be drafted to assist in such a thing, since he has also worked in this field. The same is true of other Canadian colleagues.

Do you have any suggestion as to the nature of the working conferences and when it might be possible to hold them? For example, would it be desirable to bring together at one place a group which might in the aggregate be just about as big as this one but which would work in different rooms for, let's say, two days and then spend the last day, or half day, reporting to the group as a whole on what they had done?

FLETCHER: I would like to go back to the matter of the evaluation of supervoltage. We have found that one of the main problems is the design of isodose distribution patterns which are suitable and as good at one level as at another. I don't believe there is anybody better than Dr. Johns in this field. It is first of all a physics problem and at the same time it is a clinical problem.

KAPLAN: You have Warren Sinclair available to you and if you can draft Harold Johns into working with your subgroup so much the better. I would leave that up to you.

Now the question of possible dates for a working conference comes up.

NICKSON: I should like to suggest that we hold it at the time of another meeting and that we give ourselves enough time to do a little thinking.

KAPLAN: In bringing matters to a close I should like to express my very real personal appreciation for your attendance and for the tremendous amount of work that you have put into these two and one-half days. We have had our ups and downs, but this has been far healthier than a meeting filled with sweetness and light, which would have been quite unrealistic. I think that we have arrived at a better concept of what can be achieved and how it might be achieved. A good many of us are now apparently in agreement that the time is now over-ripe to try to do something along these lines.

With that I think I would accept a motion for adjournment.

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NAME
M C Johnson Jr FEB 19 1960
V. E. Johnston
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John D. Earle MAR 7 1961
John D. Earle JUL 24 1961
Samuel D. Nellis OCT 25 1961
Phil Morgan DEC 15 1961
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